THE ONTOGENY OF MK801-INDUCED LOCOMOTION IN RATS

By

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I dedicate this dissertation to my parents, John and Lurene Frantz,
who taught me to learn.

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The locomotor effects of the *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist, MK801,were investigated in developing and adult rats. Male and female rats aged 10, 20, 30 or 55-60 days were injected subcutaneously with various doses of MK801 and placed in a novel environment (automated activity monitors) for 2 h starting either immediately after drug injection (no-delay) or following a 60 min delay (delay). In the no-delay condition, MK801 induced an inverse U-shaped dose-response function with respect to locomotion; the peak in activity occurred with 0.1 mg/kg for all ages. High doses of MK801 produced ataxia. When rats 20 days of age and older were placed in the novel environment after a 60 min delay, 0.1 mg/kg MK801 induced more activity than in the no-delay condition. The delay-potentiated activity was followed by a smooth habituation-like decline in locomotion for all rats except adult females, none of which habituated substantially to the test arena. In both no-delay and delay conditions, 0.5 mg/kg MK801 produced ataxia followed by activation in 30-day-olds and adult males or

complete ataxia in adult females. In 20-day-olds, this dose increased activity in the no-delay condition but induced ataxia in the delay condition.

In order to investigate the neuroanatomical substrate of MK801-induced locomotion, MK801 was injected into the nucleus accumbens and locomotion was recorded immediately thereafter. Eleven-day-old pups responded to 3 or 10 μg MK801 with sporadic bouts of obstinate progression. Twenty-one and 31-day-old rats injected with 10 μg MK801 exhibited locomotor activation that was qualitatively similar to that induced by peripheral injections of the drug, but adult rats were not affected by that dose. The 20 μg dose induced severe ataxia in 21-day-olds but both 20 and 40 μg doses diffused into the lateral ventricles of 31-day-old and adult rats, thus possibly increasing locomotion from other brain regions. Following both peripheral and central injections, 20-day-olds were most sensitive to MK801. Whereas peripheral injections activated 30-day-old and adult females more than males, intra-accumbens injections did not differentially affect males and females. Age- and gender-dependent variations in MK801-induced locomotion may reflect maturation of limbic-motor circuitry or the biotransformation of MK801.

CHAPTER 1

INTRODUCTION

"For the better part of this century, psychiatrists and neurologists have struggled to define the neural networks whereby biologically relevant environmental stimuli are translated into appropriate behavioral responses." (Kalivas and Barnes 1993, p. xxi). The aim of the present research was to aid in this "struggle" by investigating the neural mechanisms whereby an external stimulus, the novel environment, elicits a behavioral response, locomotion. The ontological development of this behavioral response was explored by injecting both developing and adult rats with the N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine hydrogen maleate (MK801, dizocilpine) and recording their subsequent locomotor responses in automated activity monitors to which the animals were naive. Variations on the theme included several elements: 1) analyzing the effects of introducing a novel environment at the time of peak drug effect by imposing a delay between the drug injection and placement of the rat in the activity monitor, and 2) examining a neuroanatomical substrate of locomotion by injecting the drug directly into the nucleus accumbens. Thus, the ontogeny of MK801-induced locomotion was investigated.

In experiments such as these, the variables of study should be considered separately before their interaction is analyzed. Therefore, this introductory chapter

includes a discussion of the variables of present concern: the study of ontological development, glutamate activity at NMDA receptors, novelty-induced locomotion, the nucleus accumbens, and the neurobehavioral system of study. This chapter subsequently introduces the specific experiments carried out to analyze these variables. Chapter two describes the experiment on the locomotor effects of subcutaneously-injected MK801. Chapter three describes the test of locomotor responses to MK801 injected into the nucleus accumbens. Chapter four concludes the dissertation with a review of the findings, suggestions for future experiments, and a discussion of the clinical significance of the research.

The Study of Ontological Development

The motivation to study the ontological development of behavior and its neural foundations is at least two-fold. First, examining the behavior of developing animals may enable the parsing of adult behavior into units for which the controlling parameters can be identified. Likewise, identifying the steps through which the nervous system is built might reveal how the mature system is compartmentalized. For example, young rat pups do not exhibit locomotor suppression in response to low doses of D_2/D_3 dopamine receptor agonists, but adult rats do (Eilam and Szechtman 1989, Frantz et al. 1996, Van Hartesveldt et al. 1994). Neither does the nucleus accumbens of young rat pups contain D_3 dopamine receptor mRNA, whereas the nucleus accumbens of adults rats does (Stanwood et al. 1997). Therefore, it might be postulated that dopamine agonist-induced locomotor suppression in the adult rat is mediated by selective activation of the D_3 dopamine receptor. Generally, differences between the behaviors of developing and adult

animals might correlate with differences between immature and mature neural systems mediating the behaviors. Correlations often lead to the discovery of causal relationships.

The second reason to study the ontological development of behavior and its neural foundations is to understand the process of development itself. The topic is of practical as well as academic interest. Defining normal development will aid in the identification and treatment of developmental abnormalities. The earlier a problem is identified, the more likely it is that treatment will be appropriate and beneficial. Understanding normal development will also help to define critical periods for the development of particular behaviors. An interesting example is that if the expression of adaptive responses to vestibular challenge requires exposure to gravitational forces during periods of rapid development in vestibular apparati and associated muscle fibers, then human plans to inhabit both the earth and the moon would need to include provisions for exposure to gravity during the critical periods. This developmental approach to studying behavioral and neural function can be applied to a myriad of issues in psychobiology, neurology and psychiatry.

Glutamate at NMDA Receptors

Glutamate is a ubiquitous amino acid with a plethora of functions in the nervous system (Collingridge and Lester 1989, Fonnum 1984, Herrera-Marschitz et al. 1996, Kalivas and Barnes 1993, McDonald and Johntson 1990, for reviews). In addition to serving basic metabolic functions as an amino acid, glutamate has long been recognized as a promotor of cell growth and a mechanism of cell death, especially during nervous system development. More recently, glutamate has been defined as a neurotransmitter.

At least four major subtypes of glutamate receptor mediate its neural activity, but perhaps the most interesting of glutamate's functions are mediated through NMDA receptors.

Via NMDA receptors, glutamate can mediate both neural development and cell death. By promoting the activity of neurotrophic factors, glutamate enhances neuron viability, defines neuron morphology and patterns neuronal connectivity, especially across ontogeny when such developments take place at high rates. Glutamate also regulates activity-dependent synaptic plasticity and long-term potentiation, again with increased function during neural development. For example, monocular deprivation in kittens usually results in an ocular dominance shift, such that a majority of visual cortical cells respond preferentially to stimulation of the experienced eye; treatment of the visual cortex with an NMDA receptor antagonist during the time of deprivation reduces the shift in ocular dominance (Kleinschmidt et al. 1987). On the other hand, excessive activity of glutamate at the NMDA receptor is neurotoxic, especially in the developing nervous system. The biochemical maturation of this receptor is thus an interesting topic with respect to neonatal hypoxia, ischemia and perhaps even neuronal damage leading to schizophrenia (Farber et al. 1995, Olney and Farber 1995a, McDonald and Johnston 1990).

The behavioral effects of glutamate are mediated through various pathways in the central nervous system. Most notably, glutamate is transmitted in projections from the neocortex and other cortex-like tissue masses, within several nuclei of the basal ganglia, and in sensory afferents of the spinal cord. In terms of behavior in adult rats, glutamate activity at the NMDA receptor is integral to learning and memory (e.g. Morris et al. 1986, Rison and Stanton 1995), reward-associated behaviors (e.g. Burns et al. 1994, Carlezon

and Wise 1996), responses to stress (e.g. Horger and Roth 1995, Willner et al. 1992) and locomotion (e.g. Kelley and Throne 1992). Locomotion is the focus of the present research, in part because the role of glutamate in locomotion remains elusive. Glutamate release into locomotor regions in the brain, such as the nucleus accumbens, can either increase or decrease motor activity in a manner dependent on such factors as "dopaminergic tone" in the nucleus, or environmental novelty in the testing situation (e.g. Svensson et al. 1994, Wu et al. 1993a).

In the behavior of developing rats, the role of glutamate in neural growth and behavior is even less clear. In particular, the diverse functions served by glutamate in development make its role in behavior across ontogeny difficult to define. During periods of intense neural development, such as the second and third postnatal weeks in the rat pup, glutamate and NMDA receptors are markedly abundant in the rat brain. High concentrations of glutamate or NMDA receptors do not necessarily indicate that the transmitter has behavioral consequences, however. Glutamate could serve neurotrophic function, behavioral function, or both, at any particular developmental stage.

In fact, the behavioral relevance of changing patterns in neurotransmission must be defined cautiously for any neurotransmitter. Changes in transmitter release may or may not have behavioral consequences, depending on concomitant changes in receptors or other neurotransmitters. Synaptic receptors can change across ontogeny and throughout adulthood with respect to their distribution, affinity, associated intracellular mechanisms, rates of expression and ratio of subtypes. The relevance of all of these changes to behavior should be investigated separately, as well as in a unified manner. These issues can be addressed with a variety of experimental approaches. For example,

injecting a receptor antagonist will block the function of an endogenous compound and the behavioral response to the drug should be opposite the behavioral effect of the endogenous compound. Conversely, a classic receptor agonist will activate a receptor and the behavioral response should resemble that induced by the endogenous compound. Yet, agonist injection indicates merely that the receptor has the capacity to influence behavior, not necessarily that the endogenous compound does so. This concept is especially important in studying drug effects in developing animals because receptors could become functional earlier in ontogeny than they are affected by endogenous transmitters. Thus, the study of neurotransmitters, especially glutamate, in the ontogeny of behavior poses interesting questions that require thoughtful procedures and careful interpretations to produce their answers.

Novelty-Induced Locomotion

Novelty is a major influence on behavior (O'Keefe and Nadel 1978, for review). As a quality of items or places not before experienced, novelty implies that an item or place does not yet have a representation or value in the nervous system. The behavioral effects of novelty have been investigated most often by presenting novel objects or foods in a familiar location or by placing experimental subjects in an entirely novel environment. For adult rats in a novel environment, the typical reaction is a brief period of inactivity, or freezing, followed by exploration, consisting mainly of locomotion, sniffing and rearing. Exploratory behaviors may serve to gather information about and assign biological value to stimuli in the environment. If biological value in the form of threat or reward is discovered, then appropriate goal-directed behaviors usually follow. If

biologically neutral stimuli are identified, then exploration ceases and is replaced with resting functions. The decrement in active responding to novelty is known as habituation.

The present studies concern locomotion modulated by both environmental novelty and the injection of MK801, the NMDA receptor antagonist. Separately, either of these stimuli, environmental novelty or MK801 can elicit locomotion. In a novel environment, even without drug injection, rats exhibit exploratory locomotion followed by habituation, whereas in a familiar environment without drug injection, rats do not locomote to any significant extent. After MK801 injection, whether the environment is novel or familiar, rats can exhibit high levels of exploration-like locomotion followed by an habituation-like decline in activity. In the present studies, rats were naive to the testing arena in which their locomotor behavior was measured, so the environment was novel to them. Vehicle -injected control groups therefore exhibited novelty-induced exploratory locomotion followed by habituation, while MK801-injected rats exhibited locomotion modulated by the interaction between novelty and MK801.

Increases in a behavior that appears to be exploratory in nature could represent a number of different underlying psychological events: 1) increased exploration, 2) decreased habituation of exploration, 3) decreased fear of novelty, 4) increased general motor activity, or 5) increased reactivity to stimuli. Similarly, motor activation induced by a drug injection or a lesion may reflect different underlying events. It may be different from exploration induced by novelty because it is not necessarily precipitated by a lack of information (i.e. it can occur in a familiar environment) and does not necessarily supply further information (i.e. it can continue in a repetitive, stereotypic fashion without any apparent function). Yet drug- or lesion-induced activation may have the same

topography as exploration. On the other hand, locomotor suppression could similarly represent different events, such as increased habituation or motor incapacitation. For example, the distance travelled by rats given a low dose of MK801 could be minimal because subjects slept in a corner throughout most of the test session. The distance travelled by rats given a high dose of MK801 could also be low, but perhaps because the animals were rendered ataxic to the point of akinesia. In cases of either locomotor activation or suppression, experimenter observation can aid in describing the behavior but cannot determine the motivation behind it. Precise descriptions of behavior can nevertheless help in discerning its neural substrates.

Locomotor responding to a novel environment changes across ontogeny. Exploration in response to a novel environment appears first, at low levels, in 10-day-old rat pups. Levels of exploration rise rapidly to a peak in 15-day-olds, but fall again to adult levels by 25 days of age (Campbell et al. 1969, Spear and Brake 1983). In the home cage, rat pups begin exploring late in the second postnatal week and levels of general activity in the home cage rise monotonically throughout ontogeny (Bolles and Woods 1964). Across ontogeny, male and female rats exhibit similar responses to environmental novelty, but in adulthood females are often more motorically responsive to novelty than males (Archer 1975, Campbell et al. 1969, Van Hartesveldt 1997, but see Leret et al. 1994).

At one time, responses to novelty were considered to result from competition between two elicited states within the animal, curiosity and fear (Montgomery 1955). Current vestiges of this concept can be recognized in the definition of novelty as a minor stressor and novelty-induced locomotion as a stress-response. The classification of

novelty as a stressor is substantiated by recordings of adrenocorticotropin and corticosterone release after exposure to a novel environment. The rates of increase and peak plasma levels are the same in response to novelty as to classic stressors, such as ether anesthesia (Brett et al. 1983). Gender differences in stress hormone release are also parallel in response to classic stressors and novelty. Females exhibit higher baseline levels of hormone release and the increased release due to either classic stressors or environmental novelty is higher for a longer period of time in female rats than in male rats (Brett et al. 1983, Kitay 1961). Exogenously administered corticosterone stimulates locomotor responding to novelty (Oitzl et al. 1994, Sandi et al. 1996). Conversely, corticotropin releasing factor decreases activity in a novel environment when administered via intra-cerebroventricular or subcutaneous injection, but that response is probably mediated by direct action of the releasing factor on brain receptors (Hennessy et al. 1997, McInturf and Hennessy 1996, Britton et al. 1982, Sutton et al. 1982). Behavioral responses to novelty thus occur with changes in hormone release and can be modulated by stress hormones, supporting classification of novelty-induced locomotion as a stress response.

The Nucleus Accumbens

The nucleus accumbens is known as a major site of limbic-motor interface in the brain due to its integral role not only in locomotor activity but also in reward-related behaviors and responses to stress (Mogenson et al. 1993). The nucleus accumbens lies in the forebrain as part of the ventral striatum. It receives glutamate projections from the limbic neocortex, amygdala and hippocampus (Heimer et al. 1993, for review), three limbic brain regions also involved in responses to novelty and stress (Burns et al. 1996,

Feenstra et al. 1995, Hooks and Kalivas 1994, Horger and Roth 1995, Moghaddam 1993). The nucleus accumbens receives dopamine projections from the ventral tegmental area, a group of nuclei in the midbrain motor region (Heimer et al. 1993, for review). The nucleus accumbens is consequently in a prime anatomical position to integrate several hypothesized types of input: 1) sensory information from the neocortex; 2) biological value assigned to stimuli in the amygdala; 3) comparative cognitive mapping information from the hippocampus; and 4) motor stimulation from the ventral tegmental area. Efferent projections from the accumbens target limbic areas such as the septal nucleus, bed nucleus of the stria terminalis, hypothalamus and ventral tegmental area, as well as motor nuclei such as the ventral pallidum, entopeduncular nucleus, substantia nigra and mesencephalic reticular formation. Through these outputs, the nucleus accumbens aids in translating stimuli into action.

The nucleus accumbens is likely to mediate locomotor responses to a minorly stressful stimulus, such as novelty. This prediction has experimental support. Glutamate and dopamine levels in the nucleus accumbens rise during exposure to either novelty or stress (Abercrombie et al. 1989, Bradberry et al. 1991, Hooks et al. 1992, Horger and Roth 1995, Moghaddam 1993, Rebec et al. 1997). In addition, levels of another excitatory amino acid, aspartate, rise in the nucleus accumbens in response to stress (Moghaddam 1993). Like glutamate, aspartate activates NMDA receptors, but little is known about the activity of aspartate separate from that of glutamate. Behaviorally, when locomotion in adult male rats is induced by the presence of novel objects, intra-accumbens injections of the D_2/D_3 dopamine receptor agonist, quinpirole, decrease the novelty-dependent behavior. The same doses of quinpirole injected into the nucleus

accumbens increase locomotion in a familiar environment, indicating that the effect of the dopamine agonist in the nucleus accumbens depends on the novelty of the testing environment. In developing rats, the involvement of the nucleus accumbens in novelty-induced locomotion is not adult-like until after 10 days of age, for example quinpirole does not suppress novelty-induced locomotion in 10-day-olds rats as it does in rats 20 days of age and older (Frantz and Van Hartesveldt 1995). The mechanisms within the nucleus accumbens that facilitate the role of that nucleus in response to novelty have yet to be defined but analysis of ontological changes in behaviors mediated by the nucleus accumbens may aid in that endeavor.

The System of Study

Van Hartesveldt (1995) described an ideal system to study in the context of neurobehavioral development; it is a system that modulates very simple motor behavior and that undergoes extensive postnatal development. She was referring to the mesostriatal dopamine pathway, but the same can be said of the meso-accumbens dopamine projections and cortico-accumbens glutamate projections which converge in the nucleus accumbens. Postnatal development is likely to be extensive in these pathways; dopamine receptors in the nucleus accumbens do not reach adult levels until at least postnatal day 16 (Rao et al. 1991, Stanwood et al. 1997) and there are preliminary indications that glutamate function in the nucleus accumbens does not mature until the second or third postnatal week (Campochiaro and Coyle 1978, McDonald and Johnston 1990, Subramaniam and McGonigle 1994), although the topic has not been extensively researched. The pathways converging in the nucleus accumbens certainly can modulate simple motor behaviors in developing rats, including locomotion (Frantz and Van

Hartesveldt 1995). While the components of locomotion can be complex, the use of automated activity monitors in conjunction with experimenter observation results in a simple record of activity.

Van Hartesveldt (1995) also noted that the study of mesostriatal dopamine transmission is an ideal system of study because it addresses the encephalization of control over behaviors that are exhibited from a very early age. Research on the role of nucleus accumbens glutamate in locomotion across ontogeny addresses the encephalization of motor control even more directly, because glutamate projections into the nucleus accumbens come mainly from the limbic neocortex and the cortex-like tissue masses of the hippocampus and amygdala. Encephalization of control over motor behavior classically referred to the idea of J. Hughlings Jackson that with maturation, the neocortex gradually inhibits the excitatory activity of the hindbrain motor regions (e.g. Campbell et al. 1969). The idea is now considered too simplistic, but a modified version is conceivable: with maturation, the cortex modulates subcortical motor function to an increasingly greater extent, enabling increasingly complex stimuli to either suppress or activate behavior stimulated by mid- and hindbrain motor regions. Charting the encephalization of control over the nucleus accumbens may eventually define how cortical structures modulate not only locomotion but also reward-related behaviors.

Research Questions

Within this theoretical framework, several experiments were designed to investigate the role of glutamate in locomotion across ontogeny. The specific research questions are listed below, followed by possible experimental outcomes.

1. What are the locomotor responses of developing and adult rats following subcutaneous injection of MK801 and immediate placement in a novel environment?

Behavioral responses to a glutamate receptor antagonist, MK801 should reveal the function of endogenous glutamate in locomotion. The use-dependent nature of MK801, as an open-channel blocker that halts ion flux already initiated by glutamate, enables MK801 to reveal the function of endogenous glutamate more precisely than classic receptor antagonists.

Studies of the locomotor effects of MK801 in developing and adult rats provide comparative literature for the present study. Basically, MK801 induces an inverse U-shaped dose-response function with respect to locomotion in the adult rat (Carlsson and Carlsson 1989, Ford et al. 1989, Hargreaves and Cain 1992, Tricklebank et al. 1989). Low doses of the drug do not affect behavior; mid-range doses induce hyperlocomotion, stereotyped sniffing, and turning; high doses induce ataxia which consists of body rolling, a lack of limb coordination and decreased rearing. With time after injection of high doses or immediately following injection of even higher doses, the ataxia can progress into a loss of postural support, flattened posture, footsplay, akinesia, salivation and lacrimation. A similar dose-response curve has been recorded for rat pups at several different ages, but the magnitude of activation is lower for younger animals (3-4 or 10-12 days of age) than older animals (17-18 days of age, Rajachandran et al. 1991, Scalzo and Burge 1994). The objective of this portion of the present experimentation was to extend the ontological analysis of MK801-induced locomotion over a wider age-range, longer test sessions and more consistent behavioral measures across ontogeny than had been tested previously.

There were several possible outcomes from this experiment. The locomotor effects of MK801 across ontogeny could entail the same types of drug-elicited behavior at all ages but could differ in magnitude with age. Differences across ontogeny would provoke ideas on the maturation of glutamate receptors, functional coupling between glutamate and dopamine, development of output circuitry from the nucleus accumbens or differential effects of novelty across ontogeny. Biochemical changes in glutamate transmission would then be sought out for correlation with periods of increased or decreased drug-effect. If the locomotor effects of MK801 were the same in developing and adult rats, then the transient changes in glutamate transmission that have been reported so far would presumably not have behavioral consequences, at least with respect to locomotion.

Changes in locomotion over time after drug injection could be attributed to several simultaneous events: 1) a decline in novelty of the testing environment as rats explored the test monitor, 2) a change in drug effect as MK801 was distributed and subsequently metabolized in the central nervous system, or 3) different behavioral effects of the metabolites of MK801. These possibilities would not be differentiated in the above procedure.

2. Are the locomotor effects of MK801 affected by a delay between drug injection and placement in the novel environment?

In attempt to differentiate which time-dependent changes in environmental novelty and MK801 metabolism elicited the locomotor effects in the above experiment, the interaction between MK801 and environmental novelty was investigated by imposing a 60-minute delay between the drug injection and placement of the rats in the novel

environment. The novel environment was thus introduced at a time when MK801 should have been fully distributed throughout the nervous system.

MK801 and environmental novelty could interact to potentiate or suppress the locomotion induced by either stimulus alone. If the result of the interaction were locomotor potentiation, then environmental novelty and MK801 might each increase locomotion via different neural mechanisms that are able to produce additive or synergistic effects on locomotion when combined. If the result were locomotor suppression, then the interaction between the two stimuli would have reversed the locomotor effects of each stimulus alone, indicating that the neural mechanisms counteract each other in the nervous system.

The result of the interaction between environmental novelty and fully-distributed MK801 could be consistent across ontogeny or could change with age. Consistent effects across ontogeny would indicate that the limbic-motor integration circuitry was fully functional early in ontogeny. Age-dependent changes would indicate that some aspect of the circuitry was immature early in ontogeny and characteristics of the ontological differences in responding would be used to identify corresponding neurochemical immaturities.

3. What are the locomotor effects of MK801 when injected directly into the nucleus accumbens of developing and adult rats?

Following peripheral injections, MK801 could act at NMDA receptors throughout the nervous system. In adult rats, the locomotor activating effects of systemically-administered MK801 are mediated in part by neural activity in the nucleus accumbens (Hamilton et al. 1986, Ouagazzal et al. 1994, Willins et al. 1993). Drug injections

directly into the nucleus accumbens could provide a more precise measure of activation mediated by this region. No previous investigations have addressed this issue in developing rats.

Again, the locomotor effects of MK801 could be either consistent or variable across ontogeny. In the case of variability across ontogeny, maturational changes in glutamate transmission that mediate the behavioral changes could be localized to the nucleus accumbens and efferent projections from that region. The locomotor effects of intra-accumbens MK801 could also be either consistent or variable in comparison with the effects of subcutaneously-administered MK801. Consistency in the responses would indicate that MK801-induced neural activity in the nucleus accumbens was at least sufficient to induce locomotor effects similar to the effects of peripherally-injected MK801. Variance in the responses would reflect a substantial influence of NMDA receptors elsewhere in the nervous system on locomotion induced by systemic MK801.

4. Is the injection site anatomically specific to the nucleus accumbens?

While direct intracerebral drug injections are theoretically more attractive than systemic injections for studies of neurotransmission in particular brain regions, logistically they can be difficult to carry out. For example, the drug can diffuse into brain regions surrounding the target region and is most likely to diffuse back up the track made by the injection needle. In order to avoid this confound, small injection volumes of 0.25 µl are usually used in this laboratory. The insolubility of MK801 mandated that a higher injection volume be used for higher doses of the drug administered to rats 20 days of age and older, however, increasing the likelihood of drug diffusion in the present study. If the

solution diffused, it would most likely spread dorsally into the striatum or even into the lateral ventricles. Locomotor responses would then be expected to resemble responses to intrastriatal or intra-ventricular injections of the drug. Therefore, locomotor responses to injections of MK801 into mid- and dorsal-striatal regions were recorded in 20- and 30-day-old rat pups and locomotor responses to intra-ventricular injections of MK801 were recorded in adult rats. Ten-day-old pups and adults were not tested in this experiment.

MK801 injected into the dorsal striatum of adult rats fails to produce locomotion of as high a magnitude as intra-accumbens injections (Al-Khatib et al. 1995). The same could be true of developing rats, indicating that the limbic-motor circuitry is precise as soon as it is functional. Alternatively, the specificity of the MK801 injection site could increase with age, reflecting an increased precision in glutamate circuitry as the animals mature. The time course of locomotion induced by MK801 in the various injection sites might lead to predictions about the drug's site of action. For example, intra-accumbens MK801 could increase locomotion with a long latency, but intra-ventricular MK801 could increase locomotion immediately after the injection. It might be the case, then, that the effect of intra-accumbens MK801 was mediated by the drug's activity in other brain regions to which it had diffused via the lateral ventricles.

Furthermore, the insolubility of MK801 also required its dissolution in acid before dilution to the appropriate injection volume. This procedure resulted in a final pH of approximately 4.85 for the injection solution. The acidity of the solution could induce behavioral effects itself or could affect histological staining of the tissue subsequent to the experiments. The possibility that an acidic solution could induce a behavioral effect was investigated by injecting an acidic solution without MK801 and recording

subsequent locomotor effects. Differences between responses to the MK801 solution and the acidic solution would presumably reflect the effects of MK801 on locomotion.

5. Is the ketamine/xylazine anesthesia used during cannulae implantation surgery likely to influence locomotor responding to MK801 the following day?

Ketamine was one component of the anesthesia used during cannula implantation 24 h prior to locomotor testing of intra-accumbens MK801 injections. Ketamine exerts its pharmacological action at the same site as MK801, the phencyclidine binding site inside the NMDA receptor-associated ion channel. Ketamine treatment 24 h prior to MK801 administration could therefore influence responding to MK801. Ideally, a different anesthesia could be used for the surgery, but the ketamine/xylazine compound has proven the most effective of several anesthetic compounds for experimentation with rat pups in our laboratory. Thus, a test of the effects of ketamine/xylazine pretreatment on locomotor responses to peripherally-injected MK801 was carried out in 20- and 30-day-old rats.

The anesthesia pretreatment could affect subsequent responses to MK801 in several ways. If it increased locomotion, then either a sensitization to the locomotor-activating effects of MK801 or a tolerance to the ataxia-inducing effects of MK801 would be postulated. The converse hypotheses would be proposed if the anesthesia pretreatment reduced MK801-induced locomotion. These results would indicate that the locomotor response to intra-accumbens MK801 in the previous studies may have been influenced by the anesthesia used for surgery the day before testing. It should be noted, however, that the test injections of MK801 in this test were subcutaneous, so the possibility remains that test injections directly into the nucleus accumbens would not be affected in the same

manner. For example, changes in locomotor responses to subcutaneously-injected MK801 after ketamine pretreatment could be mediated by a change in liver metabolism of MK801.

6. Do the locomotor responses to MK801 differ between male and female rats?

Both male and female rats were tested in all of these experiments for several reasons: 1) recording behavioral manifestations of interactions between glutamate and the sex hormones could facilitate better understanding of glutamate's role in locomotion and responses to stress; 2) gender-dependent drug effects have important implications for the use of pharmacological compounds in the clinic where men, women, boys and girls all need to be treated for disorders potentially related to glutamate transmission; and 3) previous experiments in adult rats have demonstrated a higher sensitivity of female rats to the locomotor effects of MK801.

If gender differences occurred at every age tested, then the differences would presumably be due to organizational effects of the sex hormones that caused permanent differences in NMDA receptor function. If gender differences showed their onset just before or during puberty (approximately 35 days of age in the rat), then the differences would more likely reflect activational effects of the sex hormones. Furthermore, sex differences might be noted in response to peripheral injections but not intra-accumbens injections, in which case they could reflect either sex differences in the liver metabolism of MK801 or effects of MK801 in other regions of the central nervous system.

7. What is to be learned from this series of experiments?

The answers to these six experimental questions should provide a solid analysis of the ontogeny of MK801-induced locomotion. The experimental results should provoke

hypotheses as to the neural mechanisms whereby environmental stimuli elicit locomotor responding and should lead to further experimental questions about the translation of motivation to action.

CHAPTER 2

LOCOMOTION ELICITED BY MK801 IN DEVELOPING AND ADULT RATS: TEMPORAL, ENVIRONMENTAL, AND GENDER EFFECTS

Introduction

The excitatory amino acid neurotransmitter, glutamate, plays an integral role in locomotor behavior. In adult rats, a well-described motor syndrome results from blockade of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor with peripheral injections of the antagonist, MK801 (Carlsson and Carlsson 1989, Ford et al. 1989, Hargreaves and Cain 1992, Tricklebank et al. 1989). MK801 is a non-competitive, use-dependent antagonist which binds to the phencyclidine (PCP) site inside the NMDA receptor-associated ion channel to block the channel after it is opened by agonist activity at the NMDA receptor. With increasing doses of the compound, the MK801-induced motor syndrome includes: 1) motor activity characterized by forward locomotion, sniffing, turning and rearing; 2) motor stereotypies such as stereotyped sniffing, head weaving and reciprocal forepaw treading; 3) ataxia, which consists of body rolling, a lack of limb coordination and decreased rearing, and 4) debilitating ataxia, which entails a loss of postural support, flattened posture, footsplay, akinesia, salivation and lacrimation. The locomotor-activating effects of MK801 endure for at least 2 h post-injection.

In developing rats, two reports have shown that subcutaneous injections of MK801 produced an adult-like inverse U-shaped dose-response curve with regard to

locomotor activity, but specific characteristics of the motor effects differed across ontogeny (Rajachandran et al. 1991, Scalzo and Burge 1994). As in adult rats, doses of 0.1 and 0.2 mg/kg MK801 increased motor behavior, but higher doses of 0.5 and 1.0 mg/kg MK801 produced ataxia in both preweanling (3-4 or 12 days of age) and weanling rat pups (17-19 days of age). The magnitude of locomotor activation was higher in weanling rats than preweanlings, but the activity of weanlings was still lower than that of adult rats (Rajachandran et al. 1991). The activity of rat pups differed from that of adult rats further in that pups did not exhibit sniffing, but adult rats do. Rajachandran et al. (1991) also reported an increase in grooming in 3-4-day-old pups given 1.0 mg/kg MK801. This activational effect was surprising, given that the same dose decreased activity (mouthing in the presence of milk) in pups of the same age and elicits akinesia in adult rats. The ataxia-producing effects of MK801 appeared to be more similar than the locomotor activating effects in adult rats and pups. In order to measure ataxia, Scalzo and Burge (1994) used a rating scale derived from that of Sturgeon et al. (1979) for PCP-induced ataxia in adult rats; PCP and MK801 bind at the same pharmacological site and induce a similar behavioral syndrome. According to the rating scale, both preweanling and weanling rats exhibited levels of MK801-induced ataxia similar to those of adult rats given the same dose. The ataxia showed no sign of decline 1 h post-injection in weanling pups.

Further comparisons between rat pups of various ages are required to determine whether or not the locomotor activating effects of MK801 rise steadily into adulthood and how locomotor responses of developing rats change with time after drug injection. Such comparisons are difficult to make based on the two studies currently available.

Rajachandran et al. (1991) observed activity for 5 min either 30 or 60 min post-injection in rats at postnatal day 3-4 and 17-18. Scalzo and Burge (1994) recorded activity for 30 min beginning 30 min after drug injection in rats at postnatal day 12 and 19 and reported time-dependent effects only for ataxia in 19-day-olds.

The first objective of the present study was to extend these developmental analyses of the locomotor effects of MK801 to include a wider age-range, use a longer testing session and record the same measure of activity in rats of various ages. Thus, MK801 was administered subcutaneously to 10-, 20-, 30-day-old and adult (54-68-day-old) rats and locomotor responses were recorded for 2 h in automated activity monitors to which the animals were naive. Under these conditions, changes in locomotor activity over time after drug injection could be attributable to two main factors: 1) the distribution and subsequent metabolism of MK801 in the nervous system and 2) a decline in novelty of the testing environment as animals could explore and habituate to the testing arena.

Environmental novelty is a major influence on locomotor activity. It elicits a locomotor response characterized in adult rats by a brief period of inactivity, or freezing, followed by exploratory behavior, consisting mainly of locomotion, sniffing and rearing, and ending with a decrement in activity, known as habituation (O'Keefe and Nadel 1978, for review). Novelty-induced locomotion changes across ontogeny (Campbell et al. 1969, Spear and Brake 1983). Exploration of a novel environment appears first at approximately 10 days of age, but in low magnitude. Levels of novelty-induced activation rise quickly to a peak at 15 days of age, but decline again to adult levels by 25 days of age. This response to a novel environment may be considered a stress response;

in adult rats, it is accompanied by release of the stress hormones, adrenocorticotropin and corticosterone, in patterns similar to release in response to classic stressors, such as ether anesthesia (Brett et al. 1983).

Responses to stress and novelty involve glutamate projections in the brain. For instance, glutamate levels in the medial prefrontal cortex, striatum, nucleus accumbens, and hippocampus rise following restraint stress or swimming stress (Moghaddam 1993). Furthermore, lesions of the medial prefrontal cortex, hippocampus or amygdala alter approach and consummatory responses to a novel food (Burns et al. 1996) and these three brain regions contain glutamatergic projection neurons. Lesions of the nucleus accumbens also alter responses to novelty (Burns et al. 1996) and the nucleus accumbens receives the glutamatergic projections from the medial prefrontal cortex, amygdala and hippocampus. Finally, injections of an NMDA receptor antagonist into the nucleus accumbens alter locomotor exploration of novel objects (Maldonado-Irizarry and Kelley 1994). Therefore, glutamate is integral not only in locomotion but also in responding to novelty and stress.

The role of glutamate in the ontogeny of novelty-induced locomotion is unknown. Therefore, the second objective of the present study was to analyze the interaction between environmental novelty and MK801 in eliciting locomotion in developing and adult rats. MK801 was injected subcutaneously as in the previous condition, but the rats were not placed in the activity monitors until 60 min after the injection. The novel environment was thus presented at the time of peak drug effect, defined as the time of highest locomotor activation under the condition without a delay between drug injection and placement in the activity monitor.

Previous research has shown that adult female rats exhibit higher hormonal and behavioral responses than male rats to environmental novelty (Archer 1975, Campbell et al. 1969, Van Hartesveldt 1997), stress (Brett et al. 1983, Kitay 1961) and MK801 (Fleischmann et al. 1991, Hönack and Löscher et al. 1993, Blanchard et al. 1992, Haggerty and Brown 1996). Therefore, both male and female rats were tested in the present studies.

Materials and Methods

Subjects

Experimental subjects were derived from Sprague-Dawley dams and sires obtained from Zivic-Miller Laboratories, Inc. (Portersville, PA). Female rats were placed in breeding cages with males and were given daily vaginal lavage to check for sperm. Pregnant females were checked twice daily for litters, so that the time of birth was recorded within 12 h. The day of birth was recorded as day 0. On day 1, litters were culled to 10 pups with approximately equal numbers of males and females. On day 25, the rat pups were weaned, separated by sex and housed with at least one other littermate. Colony rooms were maintained at 21° C on a 10:14 light:dark cycle with lights on at 08:00 h. Testing took place between 08:00 and 18:00 h in a room maintained at 26° C.

Rat pups were tested at 10, 20 and 30 days of age. Adult rats were between 54 and 68 days of age. Each dose-group consisted of 8 to 15 rats with approximately equal numbers of males and females in each group. On the day of testing, a split-litter design was used so that at least three doses of the drug were tested in each litter. Each rat was tested only once.

Drug Procedure

The glutamate NMDA receptor antagonist, (+)MK-801 hydrogen maleate (dizocilpine maleate, Research Biochemicals International, MA, USA), was dissolved in saline, which was also used as the control injection. Drug solutions were prepared on the day of testing. Subcutaneous injections were administered at the nape of the neck in doses of 0 mg/kg (vehicle injection), 0.01, 0.10, 0.50 or 1.00 mg/kg. Injection volumes were 2.5 ml/kg for rat pups and 1.0 ml/kg for adult rats. These doses were equivalent to 0, 0.0296, 0.296, 1.48 or 2.96 μmol/kg. Severe ataxia occurred in 10-day-old pups given 0.5 mg/kg MK801. The highest dose (1.0 mg/kg) was therefore not administered to this age-group in any condition. Because the highest doses administered in the no-delay condition induced severe ataxia in animals of all ages, these doses (0.5 mg/kg for 10-day-olds and 1.0 mg/kg for older animals) were not tested in the delay condition.

Behavioral Procedure

On the day of testing, rats were transported in their home cages from the colony rooms to the working laboratory. Ten- and 20-day-old rats were maintained with their dams until testing. For the condition without a time delay (no-delay), injections were given in the testing room and recording began immediately after the injection. For the delay condition (delay), drug injections were given in a room separate from the testing room. After the injection, rats were returned to their home cages with littermates (and dam if pups were 10 or 20 days of age) but were kept in the laboratory. One h later, each rat was transported for the first time into the testing room and was placed in a randomly assigned activity monitor for a 2 h test session.

Omnitech Digiscan Animal Activity Monitors recorded the locomotor activity of the rats. Each monitor was a 41.91 cm x 41.91 cm x 30.48 cm Plexiglas cage with a wire mesh floor. Photocell beams projected across the arena. They were spaced 2.54 cm apart such that 16 beams crossed side to side and 16 beams front to back, all 3 cm above the mesh floor. Solid flooring was added for the 10-day-old rats, so that beams crossed 1.5 cm above this floor board. The interruption of photocell beams was translated into various measures of locomotor activity by the Digiscan analyzer. Total distance travelled in cm was analyzed in 5 min intervals over a period of 2 h. Experimenter observation of locomotor behavior was carried out simultaneously with the automated measure of locomotion, in order to gauge qualitatively the various motor behaviors.

Statistics

Separate three-way analyses of variance with repeated measures (time factor) were carried out for each age group and each delay condition with drug dose, gender and time interval as the main factors. Additional analyses were conducted to compare groups in the delay and no-delay conditions, such that four-way analyses of variance included drug dose, gender, time interval and delay condition as factors. Follow-up comparisons were made using three-way, two-way and one-way ANOVAs followed by Duncan's New Multiple Range Test to determine significance at the $P < 0.05$ or $P < 0.01$ level.

Results

No-Delay Conditions

MK801 induced an inverse U-shaped dose-effect curve with respect to locomotion (Fig. 2-1). In rats of all ages tested, the lowest dose did not alter the distance travelled compared with the saline-injected control group, mid-range doses increased activity and

higher doses induced ataxic effects. The locomotor activation consisted of hyperlocomotion, stereotyped sniffing, "frantic exploration" of the test monitor, hyper-reactivity and hyper-excitability. The ataxia consisted of head weaving, reciprocal forepaw treading, body rolling and decreased rearing. With higher doses, the motor response progressed into debilitating ataxia, involving a flattened posture, footsplay, immobility, akinesia, salivation and lacrimation. The quality of activity resembled that described by Sturgeon et al. (1979) who designed a rating scale for locomotor activity, stereotypy and ataxia induced by PCP, a compound active at the same binding site as MK801. In terms of the dependent measure in the present study, distance travelled in cm, only the mid-range doses of MK801 robustly increased the distance travelled. Neither the lowest nor highest doses significantly changed the measure, but for different reasons. The lowest dose did not alter behavior. The highest doses elicited severe ataxia to the point of akinesia, bringing the distance travelled down to the level of vehicle-injected control rats. Ten-day-old vehicle-injected rats exhibited low levels of activity for the first 5 min of the test session; vehicle-injected rats of other ages exhibited locomotor exploration of the novel environment followed by habituation within approximately 20 min (Fig. 2-2).

Ten-day-old rat pups

In 10-day-old rat pups, MK801 affected the total distance travelled over the 2 h test session in a dose-dependent manner (Fig. 2-1). The 0.1 mg/kg dose significantly increased the total distance travelled, as revealed by a significant main effect of dose [$F(3,35)=5.49$, $P<0.001$]. The 0.01 mg/kg dose had no effect on distance travelled and 0.5 mg/kg MK801 produced akinesia, resulting in a level of activity in the range of the

control group. The activity elicited by 0.1 mg/kg MK801 included inconsistent, sporadic bouts of motor activity, sometimes including ataxic behaviors such as forepaw treading and body rolling. When the data were analyzed in 5 min intervals over the 2 h test session, the variability across individual rat pups obscured statistical significance of the dose x time interaction [$F(69, 805)=1.18$] (Fig. 2-2A). There were no noted differences between genders.

Twenty-day-old rat pups

In 20-day-old rat pups, MK801 again induced an inverse U-shaped dose-effect curve with respect to the total distance travelled during the entire 2 h test session (Fig. 2-1). The 0.01 mg/kg dose did not significantly alter the total distance travelled; the 0.1 and 0.5 mg/kg doses significantly increased the distance travelled ($P<0.01$) and the 1.0 mg/kg dose induced akinesia, bringing this measure down to that of the habituated control group. Thus, the main effect of dose was significant [$F(4,49)=16.27$, $P<0.001$]. There were no noted differences between genders.

The total distance travelled in 5 min intervals was significantly different according to a dose x time interaction [$F(92,1127)=2.786$, $P<0.001$] (Fig. 2-2B). The 0.1 mg/kg dose consistently increased the distance travelled from 25-105 min post-injection ($P<0.01$ at 25-75 min and 85 min intervals, $P<0.05$ at 80, 90, 100 and 105 min intervals). The 0.5 mg/kg dose also consistently increased the distance travelled from the 15 min interval through the end of the test session ($P<0.01$). Rats injected with the low (0.01 mg/kg) or high (1.0 mg/kg) doses of MK801 did not exhibit significantly different activity levels, compared with vehicle-injected rats.

Thirty-day-old rat pups

In 30-day-old rat pups, MK801 induced an inverse U-shaped dose-effect curve (Fig. 2-1). The 0.01 mg/kg dose did not significantly alter the total distance travelled. The 0.1 mg/kg dose significantly increased the distance travelled ($P<0.01$). The 0.5 mg/kg dose increased activity but not significantly. The 1.0 mg/kg dose induced akinesia, bringing the activity level back down to that of the control group. In a two-way ANOVA with gender and dose as factors, there was no significant effect of gender [$F(1,44)=0.9008$], nor was the gender x dose interaction significant [$F(4,44)=1.48$], although females were slightly more active than males (Fig. 2-1 inset). The main effect of dose was also significant [$F(4,44)=7.47$, $P<0.001$] (Fig. 2-1).

The total distance travelled in 5 min intervals was significantly different according to a three-way gender x dose x time interaction [$F(92,1012)=1.29$, $P<0.05$] as well as a significant time effect [$F(23,1012)=18.56$, $P<0.0001$] (Fig. 2-2C). Male and female rat pups injected with the low (0.01 mg/kg) dose of MK801 did not exhibit significantly different activity levels, compared with vehicle-injected rats. The 0.1 mg/kg dose increased the distance travelled differentially for males and females; the activity of males was increased from time interval 30-55 ($P<0.01$ at intervals 30, 35 and 45; $P<0.05$ at intervals 40, 50 and 55) and the activity of females was increased at various intervals starting at 30 min post-injection ($P<0.01$ at intervals 40 and 50, $P<0.05$ at intervals 30, 55, 60, 85, 90, 115 and 120), compared with the same-sex vehicle-injected controls. Females were more active than males at only three time intervals: 90, 115 and 120 min ($P<0.05$). The 0.5 mg/kg dose did not differentially affect males and females. For both genders, it increased locomotion at the beginning of the session ($P<0.01$ at time interval

15 min), induced akinesia from approximately 15-60 min, but increased locomotion again at the end of the session ($P<0.01$ at intervals 110, 115 and 120 min, $P<0.05$ at interval 100 min).

Adult rats

MK801 administered to adult rats again induced an inverse U-shaped dose-effect curve with respect to the total distance travelled during the entire 2 h test session, but the responses differed between genders (Fig. 2-1). In males, the 0.01 mg/kg dose did not significantly alter the total distance travelled; 0.1 and 0.5 mg/kg MK801 significantly increased the distance travelled ($P<0.05$ and $P<0.01$, respectively), and 1.0 mg/kg MK801 induced ataxia, resulting in measures of activity similar to those of the control group. In females, the 0.01 mg/kg dose did not significantly alter the total distance travelled; 0.1 mg/kg substantially increased the total distance travelled ($P<0.01$), but both 0.5 and 1.0 mg/kg induced ataxia, bringing the distance travelled down to that of the control group. A two-way ANOVA with gender and dose as factors revealed a significant main effect of dose [$F(4,41)=16.846$, $P<0.0001$] as well as a significant gender x dose interaction [$F(4,41)=10.508$, $P<0.0001$].

The distance travelled in 5 min intervals was significantly different over time [$F(23,943)=9.042$, $P<0.0001$] and according to a three-way gender x dose x time interaction [$F(92,943)=4.16$, $P<0.0001$] (Fig. 2-3). In males, the 0.1 mg/kg dose increased the distance travelled from time interval 30-45 and interval 65 ($P<0.05$), compared with the male control group. The 0.5 mg/kg dose induced akinesia which was followed by activation in male rats, such that the distance travelled was increased from time interval 75 through the end of the session ($P<0.05$ at intervals 75 and 80, $P<0.01$

through the rest of the session). In females, the 0.1 mg/kg dose of MK801 increased activity robustly from time interval 20 through the end of the session ($P<0.01$), compared with the female control group. The 0.5 mg/kg-induced akinesia was not followed by locomotor activation in females. Comparisons across gender showed that males and females did not differ from each other after the 0.1 mg/kg dose, but males injected with 0.5 mg/kg MK801 were significantly more active than females given the same dose at interval 55 and from interval 65 through the end of the session ($P<0.05$ at intervals 55 and 65-85, $P<0.01$ at intervals 90-120). Both males and females exhibited ataxia in response to 1.0 mg/kg MK801 and their activity did not differ from that of control groups. Neither male nor female rats injected with the low (0.01 mg/kg) dose of MK801 exhibited significantly different activity levels, compared with same-sex control groups.

Delay Conditions

Imposing a delay between drug injection and placement of the rat in the novel environment altered the time course and severity of locomotor responses to MK801 (Fig. 2-4 through 2-8). Qualitatively, however, the locomotor responses were similar, i.e. locomotor activation and ataxia including akinesia were the types of behavior induced by the drug. The 0.01 mg/kg dose of MK801 did not significantly alter the distance travelled in either the no-delay condition (as described above) or the delay condition (data not shown).

Ten-day-old rat pups

When 10-day-old rat pups were injected with MK801 (0.01 or 0.1 mg/kg) and placed into activity monitors 60 min later, their locomotor responses differed with respect to dose [$F(2,40)=8.335$, $P<0.001$], time [$F(23,920)=12.031$, $P<0.0001$] and the dose x

time interaction [$F(46,920)=4.995$, $P<0.0001$](Fig. 2-4). The 0.1 mg/kg dose of MK801 significantly increased the distance travelled at post-injection time intervals 65, 70, 75 ($P<0.01$) and 80 ($P<0.05$) min.

Imposing the delay between drug injection and placement in the activity monitor produced locomotor activity of lower overall magnitude than that produced in the no-delay condition. Also, the activity levels of the pups appeared to be less variable in the delay condition than in the no-delay condition both across animals at each time interval and in individual pups over time. A three-way ANOVA revealed significant effects of delay condition [$F(1,67)=5.053$, $P<0.05$], dose [$F(2,67)=8.84$, $P<0.001$] and time [$F(11,737)=1.99$, $P<0.050$], as well as a condition x dose x time interaction [$F(22,737)=1.73$, $P<0.05$]. Follow-up analyses revealed that rat pups in the delay conditions exhibited significantly less activity than those in the no delay condition at 80, 85, 95 and 100 min post-injection.

Twenty-day-old rat pups

A substantial potentiation of the initial levels of locomotor activity occurred when 20-day-old rat pups were injected with 0.1 mg/kg MK801 and placed in activity monitors 60 min later (Fig. 2-5A). On the other hand, akinesia resulted when 20-day-old pups were injected with 0.5 mg/kg MK801 and placed in the activity monitors after a 60 min delay (Fig. 2-5B).

Statistical tests confirmed the effects of the delay conditions. The three-way ANOVA revealed a significant interaction of delay condition x dose x time [$F(33.891)=2.16$, $P<0.001$]. Following 0.1 mg/kg MK801, the delay condition x time interaction was significant [$F(11,209)=3.45$, $P<0.001$] and follow-up statistics revealed

that the distance travelled in the first 5 min interval of the delay condition (65 min post-injection) was significantly higher than the distance travelled by the pups in the no-delay condition at the same time post-injection (65 min, $P<0.05$, Fig. 2-5A). On the other hand, following 0.5 mg/kg MK801, the two-way ANOVA of delay condition x time showed only a main effect of condition [$F(1,20)=15.872$, $P<0.001$] because the delay condition produced ataxia consistently throughout the test session while the pups in the no-delay condition were consistently activated through that time period (Fig. 2-5B).

Comparisons within the delay condition revealed significant main effects of dose [$F(3,40)=11.74$, $P<0.0001$], time [$F(23,920)=19.27$, $P<0.0001$] and a dose x time interaction [$F(69,920)=5.30$, $P<0.0001$]. The 0.1 mg/kg dose increased the distance travelled, relative to the saline-injected controls in the delay condition, from approximately 65-150 min post-injection ($P<0.01$ from 65-115 and 150 min, $P<0.05$ at 120, 135 and 140 min, Fig. 5A).

Thirty-day-old rat pups

As in the 20-day-old pups, a potentiation of the initial levels of locomotor activity took place when a delay was imposed between injection of 0.1 mg/kg MK801 and placement of the rat in the activity monitor (Fig. 2-6A). Interestingly, the delay conditions did not change the time course of effect for 0.5 mg/kg MK801; the drug induced akinesia at the beginning of the session, but locomotor activation ensued (Fig. 2-6B). These delay condition effects were similar for males and females.

Statistics confirmed the significance of the effects of the delay conditions. Although there was no longer a difference between genders in response to 0.1 mg/kg MK801 in the delay condition, the data were analyzed separately for males and females in

order to compare the data with the same-sexed groups in the no-delay condition (which were slightly different from each other). For males, the delay condition x dose x time interaction was significant [$F(33,407)=19.89$, $P<0.0001$] and follow-up tests revealed that the 0.1 mg/kg dose increased the distance travelled more in the delay condition than in the no-delay condition from 65-90 min post-injection ($P<0.01$ from 65-75, $P<0.05$ at 85 and 90 min, Fig. 2-6A). For females, the delay condition x dose x time interaction was also significant [$F(33,429)=22.54$, $P<0.0001$] and follow-up tests revealed that the 0.1 mg/kg dose increased the distance travelled more in the delay condition than in the no-delay condition from 65-90 min post-injection ($P<0.01$ from 65-80, $P<0.05$ at 85 min, Fig. 2-6A). In addition, females given 0.1 mg/kg MK801 in the delay condition travelled less than females in the no-delay condition at 120 min post-injection ($P<0.05$). On the other hand, the 0.5 mg/kg dose of MK801 induced ataxia followed by activation that did not differ between conditions or genders (Fig. 2-6B).

Comparisons within the delay condition revealed significant effects of dose [$F(3,44)=19.09$, $P<0.0001$], time [$F(23,1012)=18.99$, $P<0.0001$] and a dose x time interaction [$F(69,1012)=37.70$, $P<0.0001$]. The 0.1 mg/kg dose increased the distance travelled, relative to the saline-injected controls in the same delay conditions, from approximately 65-125 min post-injection ($P<0.01$ from 65-100 min, $P<0.05$ at 105-125). The 0.5 mg/kg dose suppressed the initial increase in distance travelled during the 65 min interval (the first 5 min of exposure to the activity monitor) but steadily increased distance travelled from the 95 min interval through the end of the session ($P<0.01$ except at 105 min when $P<0.05$). There were no differences between genders.

Adult rats

The potentiation of the initial levels of locomotor activity occurred again when a delay was imposed between the injection of 0.1 mg/kg MK801 and placement of adult rats in the activity monitor (Fig. 2-7A and B). Not only did the females exhibit potentiated levels of activity in the delay condition but also they continued to locomote at high rates throughout the 2 h test session. For 0.5 mg/kg MK801, the delay conditions did not change the time course of drug effect; 0.5 mg/kg MK801 induced complete ataxia throughout the session for the females but for males the ataxia was followed by locomotor activation (Fig. 2-8A and B).

Statistical tests confirmed the significance of the effects of the delay conditions. For males, the delay condition x dose x time interaction was significant [$F(33,319)=2.871$, $P<0.0001$] and follow-up tests revealed that the 0.1 mg/kg dose increased the distance travelled more in the delay condition than in the no-delay condition from approximately 65-120 min post-injection ($P<0.01$ from 65-75, 100 and 115 min intervals, $P<0.05$ at 80-90 and 120 min, Fig. 2-7A). For females, the delay condition x dose x time interaction was significant [$F(33,374)=2.089$, $P<0.001$] and follow-up tests revealed that the 0.1 mg/kg dose increased the distance travelled more in the delay condition than in the no-delay condition from 65-75 min post-injection ($P<0.05$, Fig. 2-7B).

Comparisons within the delay conditions revealed a significant effects of dose [$F(3,27)=25.79$, $P<0.0001$] and time [$F(23,621)=16.88$, $P<0.0001$] as well as a significant gender x dose x time interaction [$F(69,621)=6.58$, $P<0.0001$]. For males, the 0.1 mg/kg dose increased the distance travelled from approximately 65-90 min post-injection

($P<0.01$ from 65-75 min, $P<0.05$ at 85 and 90 min, Fig. 2-7A). For females, the 0.1 mg/kg dose increased the distance travelled, relative to the saline-injected controls in the delay conditions, at every time interval in the session ($P<0.01$, Fig. 2-7B). The distance travelled by females given 0.1 mg/kg MK801 was even greater than that of males in the same delay conditions from 70 min post-injection through the end of the session ($P<0.01$ except at 70, 75, 95, 100, 110, and 115 min intervals when $P<0.05$). Males given 0.5 mg/kg MK801 exhibited ataxia at the beginning of the session but 95 min post-injection through the end of the session ($P<0.01$ except at 95, 105 and 110 min intervals when $P<0.05$, Fig. 2-8A). This activation in males was greater than in the females given the same 0.5 mg/kg dose of MK801 from time interval 85 through the end of the session ($P<0.01$ except at 85, 105, 115 and 120 min when $P<0.05$). The 0.5 mg/kg dose induced severe ataxia in females, throughout the delayed test session, so there were no significant differences from the control group (Fig. 2-8B).

Discussion

MK801 induced an inverse U-shaped dose-response function with respect to locomotion in rats of all ages. The low dose had no effect; mid-range doses induced hyperlocomotion and stereotypies; and higher doses induced ataxia and even akinesia. The quality of these effects was similar in rats 20 days of age and older, but 10-day-old rats showed a different behavioral topography. In addition, the relative dose-responsivity to MK801 varied across age and gender such that 20-day-old pups were the most active overall and females were more active than males at 30 and 60 days of age. Introduction of a novel environment 60 min after the MK801 injection also altered the drug effects in a dose-, age- and gender-dependent manner. Variations in the motor responses to MK801

and a novel environment may reflect changes in stress hormone release, the neural circuitry mediating the effects, and the biotransformation of MK801.

Ten-Day-Old Rat Pups

Ten-day-old rat pups exhibited motor responses to MK801 and environmental novelty that differed from those of older rats. First, 10-day-old saline-injected control rats exhibited low levels of novelty-induced locomotion and they habituated to the test monitor within 5 min. This effect is in accordance with reports that exploration in response to novelty is first apparent at 10 days of age at a low level (Campbell et al. 1969), but contrasts the behavior of older pups and adults in the present study, which consisted of high initial levels of locomotion with habituation within approximately 20 min. Second, in response to 0.1 mg/kg MK801, 10-day-old pups demonstrated low levels of activation that occurred in short bouts with varying times of onset and offset for each animal. This time course of MK801-induced activation in 10-day-old rats is reported here for the first time. The low-level of activation confirms previous findings on MK801-induced activity in rat pups (Rajachandran et al. 1991, Scalzo and Burge 1994), as does the lack of sniffing behavior noted in 10-day-old pups. Third, introducing a novel environment 60 min after the injection of 0.1 mg/kg MK801 did not potentiate the initial levels of locomotion in 10-day-old rat pups, as it did in older rats. However, the delay condition did induce activity in 10-day-olds that was less variable than in the no-delay condition, such that locomotion was significantly increased above control levels by 0.1 mg/kg MK801 for the first 15 minutes after placement in the activity monitor and declined at a consistent rate across animals. Thus, the delay apparently "organized" or decreased the variability in the behavior across the group of 10-day-old pups in some

manner. Finally, the ataxia with akinesia induced by 0.5 mg/kg MK801 in the present study contrasts with the increase in grooming reported by Rajachandran et al. (1991) following injection of this dose to 3-4-day-old rats. Procedural and age differences may have produced this difference in behavior.

There are several possible explanations for the low magnitude and bout-like responses of 10-day-old rat pups to MK801. One possibility that can be ruled out, though, is that the body length of the rat mandates that shorter distances travelled reflect the same degree of activation. Rats of various ages obviously vary in body-length but total distance in cm does not account for body length. The ontological differences in magnitude of responding could not be accounted for entirely by dividing total distance by body length, however. Body length increases monotonically with age, but the magnitude of locomotor responding does not. Therefore, while the activity levels of 10-day-olds would appear closer in magnitude to those of 20-day-olds, the activity of 20-day-olds would appear even higher relative to adults, leaving the ontological changes in magnitude of response unexplained.

The behavioral repertoire of 10-day-old rat pups typically includes short bursts of activity. Spontaneous behavioral arousal of 10-day-olds in the home cage consists mainly of two behaviors: suckling and huddling. When 10-day-olds are activated by a drug in a testing situation without a dam, suckling is not an option, so the motor behavior exhibited by the pups may resemble huddling in that it occurs in short, sporadic bouts of motor behavior. Drug-induced wall-climbing in rat pups also occurs in bouts (Barrett et al. 1982). In addition, injections of the D_2/D_3 dopamine receptor agonist, quinpirole, induce locomotion in 10-day-olds that is sporadic and occurs at different times across a

test session for individual pups (Frantz and Van Hartesveldt 1995, Van Hartesveldt et al. 1994). Ten-day-old pups, however, do have the ability to exhibit more consistent high levels of locomotion than they did in the present study, as demonstrated in response to another D_2/D_3 dopamine receptor agonist, 7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin (Frantz et al. 1996). Therefore, activity in short bouts may be an age-typical response to the stimulation, but it is not the only possible response in 10-day-old pups.

In fact, bouts of activity were no longer prevalent under the delay condition. Rather, all the pups were activated consistently for the first 15 min of testing. The effect of the delay condition on locomotion in 10-day-olds may have been attributable to the activity of the pups during the first h after drug injection. In the delay condition, environmental novelty did not influence the MK801 effect, but interactions with their dam and littermates may have; the pups were placed back in their home cages immediately after drug injection. These parameters may have increased locomotion during the first h post-injection with a consistent time of activational onset across pups. The group consistency may have extended to a stable level of activation upon initial placement in the activity monitors and a subsequent simultaneous time of fatigue or habituation. The onset of activation in the home cage during the delay was not recorded, but observation of one litter revealed that locomotor activity during the first h post-injection was higher and more constant in the home cage than in the activity monitors. In the no-delay condition, the time of onset of locomotion may have been more variable than in the delay condition, due to differences across pups in responding to environmental novelty while MK801 was simultaneously being distributed throughout the nervous system during the first h post-injection. If that was the case, the result was the highly

variable level of activation during the 2 h test session for the no-delay group on the whole.

The question still remains as to why, in terms of neural mechanisms, the responses to the combine stimuli of novelty and MK801 were different in 10-day-old pups compared to older animals. Insofar as exposure to novelty is considered a mild stressor, responses to novelty and their modulation by MK801 can also be contemplated in relation to stress hormones. In adult rats, exposure to a novel environment increases plasma levels of corticosterone and adrenocorticotropin (ACTH, Brett et al. 1989). Increased plasma corticosterone may increase locomotor responding to novelty, as exogenous corticosterone injections increase locomotion in a novel environment (Oitzl et al. 1994, Sandi et al. 1996). Moreover, MK801 and stress hormones have mutually activating effects; corticosterone injection increases the locomotor activating effects of MK801 and MK801 injection increases plasma levels of corticosterone and ACTH (Pechnick et al. 1989, Wedzony and Czyrak 1994). Overall, both novelty and MK801 can increase plasma levels of stress hormones and all three stimuli can increase locomotion.

Across ontogeny in rat pups, profound changes in the release of stress hormones occur. Most notably, the "stress non-responsive period" during the first two postnatal weeks involves decreased hypothalamic-pituitary-adrenal axis responses to noxious stimuli (Walker et al. 1986). If 10-day-old rat pups do not exhibit increased levels of stress hormones in response to novelty or to MK801, then none of the interactions between novelty, stress hormones and MK801 would occur and no potentiation of locomotion would be exhibited.

Another mechanistic explanation for the low and sporadic drug response in 10-day-olds is that some aspect of the limbic-motor circuitry in the brain is not yet fully functional. In adult rats, novelty-induced locomotion and MK801-induced locomotion can be modulated by neural activity in the nucleus accumbens (Al-Khatib et al. 1995, Burns et al. 1996, Hamilton et al. 1986, Maldonado-Irizarry and Kelley 1994, Svensson and Carlsson 1992, Svensson et al. 1994, Willins et al. 1993, Wu et al. 1993b). The prefrontal cortex and hippocampus, which project glutamate into the nucleus accumbens, mature relatively late in ontogeny (Angevine, Jr. 1975, Alexander and Goldman 1978, Benes 1989, O'Keefe and Nadel 1978). It is thus conceivable that glutamatergic projections into the nucleus accumbens are not yet functional in the 10-day-old rat. On the other hand, sufficient levels of glutamate could be released, but NMDA receptors may not yet be associated with the appropriate intracellular mechanisms or may not be present on the appropriate output neurons. In this respect, the lack of consistent motor responding to MK801 or novelty could represent a functional "pre-coupling" of glutamate to the output from the nucleus accumbens. The sporadic motor response to MK801 could furthermore be mediated by blockade of NMDA receptor-associated ion channels elsewhere in the central nervous system. Thus, there are many possible explanations for the response of 10-day-olds to MK801. Given the complexity of nervous system development, several of them are likely to interact to produce ontological changes in motor responding.

Rats 20 Days of Age and Older

In rats 20 days of age and older, the initial locomotor response to novelty was greatly potentiated when a delay was imposed between the injection of 0.1 mg/kg

MK801 and placement of the rat in the activity monitor. Therefore, blockade of glutamate NMDA receptors and introduction to the novel environment interacted to increase locomotion, such that the activation was greater than activity induced by either MK801 as a stimulus alone (as shown by the MK801-injected groups in the no-delay condition) or environmental novelty as a stimulus alone (as shown by the saline-injected control groups). In addition, the initially high levels of activity were followed by a rapid habituation-like decline in locomotion, except in adult female rats that did not habituate substantially to the test monitor.

Whereas the lack of locomotor potentiation in 10-day-old pups may be attributable to stress non-responsiveness, the potentiated levels of locomotion in older animals may be attributable to an interaction between stress hormones and MK801. Rats 20 days of age and older would have passed through the stress non-responsive period and would presumably exhibit increased plasma levels of stress hormones in response to a novel environment (Brett et al. 1989). In addition, MK801 would increase plasma corticosterone and ACTH levels (Pechnick et al. 1989, Wedzony and Czyrak 1994). Since injections of exogenous corticosterone increase the locomotor activating effects of MK801 (Wedzony and Czyrak 1994), corticosterone released through exposure to novelty and MK801 might also increase locomotion, to a greater extent than when corticosterone was released in response to just one of those stimuli. Therefore, introducing the novel environment at the time of peak MK801 effect may have potentiated the initial levels of locomotion by also potentiating corticosterone release.

When the higher dose of 0.5 mg/kg MK801 was tested, the introduction of the novel environment 60 min after the injection completely changed the drug effect from

robust activation to debilitating ataxia (including loss of postural support, footsplay and akinesia) in 20-day-old pups. The effect of introducing a novel environment at the time of peak drug effect was thus similar to increasing the effect of MK801. In other words, increasing the dose of MK801 given to 20-day-old pups from 0.5 mg/kg to 1.0 mg/kg in the no-delay condition resulted in a change from locomotor activation to akinesia. The same effect occurred when novelty was added to the 0.5 mg/kg dose of MK801 in the delay condition. Again, the potentiation of the effect of MK801 on motor activity may have been mediated by an increase in stress hormone release. In 30-day-olds and adults, however, the delay condition did not change the effect of 0.5 mg/kg MK801, perhaps indicating that the drug effect was maximal at 60 min post-injection and could not be increased further by environmental novelty. These rats were, in fact, akinetic at 60 min post-injection. Further stimulation could not decrease activity any more. The rats did exhibit high levels of locomotion at approximately 90 min post-injection, perhaps induced by the interaction between what was essentially a lower dose of MK801, as the drug was metabolized, and novelty of the environment that they had not been capable of exploring while akinetic.

Whether locomotion is stimulated by stress hormone release, blockade of NMDA receptors, or an interaction between the two, the behavior is likely to be mediated by the nucleus accumbens, given that glutamate in the nucleus accumbens is known to play a major role in responses to stress. Ontological variations in sensitivity to the effects of MK801 are thus also likely to reflect changes in nucleus accumbens circuitry, generally, and in glutamate transmission, specifically. Twenty-day-old pups exhibited the highest magnitude of MK801-induced locomotion, perhaps due to high levels of circulating

glutamate release, high densities of NMDA receptors or supersensitive receptors for glutamate or other neurotransmitters in the motor circuitry. Indeed, at least in the striatum proper, several measures of glutamate functioning reach or overshoot adult levels during the second and third postnatal weeks and could mediate supersensitivity to MK801 early in their development (McDonald and Johnston 1990, for review). Similar developments in the nucleus accumbens have not yet been investigated but are certainly conceivable. Thirty-day-old pups exhibited the lowest magnitude of locomotion, significant only at the 0.1 mg/kg dose. This effect could reflect programmed cell death, synaptic pruning or receptor elimination after the growth spurt at 20 days of age. The adult levels of locomotion induced by MK801 most likely reflect the stabilization of glutamate transmission with increased maturity. While these hypothesized neural mechanisms provide an attractive explanation for the variations in drug sensitivity, peripherally-injected MK801 could have exerted its effects anywhere in the nervous system. For example, the ontological differences in responding could reflect maturation of liver metabolism of the drug. Similar ontological changes in sensitivity to MK801 following intra-accumbens injections of the drug, however, undermine the likelihood of that concept proving valid (see Chapter 3).

Gender Differences

The greater responsiveness of female rats compared with males to the motor effects of MK801 has been well-quantified (e.g. Fleischmann et al. 1991, Hönack and Löscher et al. 1993, Blanchard et al. 1992, Haggerty and Brown 1996) and is demonstrated in several ways by the present results. Females exhibited higher activation by 0.1 mg/kg MK801 and, in the delay condition, they did not habituate substantially to

the test arena following the compound stimulation of 0.1 mg/kg MK801 and the novel environment. In addition, females given 0.5 mg/kg MK801 exhibited more severe ataxia for a longer period of time than males given the same dose.

Gender differences in MK801-induced locomotion most likely reflect differences in the metabolism of MK801. Female rats have less efficient liver breakdown of PCP than males (Nabeshima et al. 1984a), a characteristic dependent upon gonadal hormones (Nabeshima et al. 1984b). Given that PCP and MK801 bind to the same site in the open NMDA receptor-associated ion channel and elicit similar behavioral effects, a similar biotransformation for the two drugs is likely. MK801 may therefore be active in the nervous system of female rats at a higher concentration for a longer period of time and consequently may exert greater behavioral effects in the form of higher activation in response to 0.1 mg/kg MK801 and longer periods of akinesia in response to 0.5 mg/kg MK801. Mediation of gender differences by peripheral mechanisms is supported by a lack of gender-dependent responses to centrally-injected MK801 (see Chapter 3). However, Hönack and Löscher (1993) point out that gender-dependent behavioral differences can occur within minutes of drug injection, undermining the importance of metabolic differences.

The gender differences in MK801- and novelty-induced locomotion may also be related to stress hormones. If females metabolize MK801 more slowly than males (Nabeshima et al. 1984a) and if MK801 increases plasma levels of ACTH and corticosterone (Pechnick et al. 1989), then a higher level of MK801-induced stress-hormone release may occur in females than males, thus inducing the high levels of MK801-induced locomotion in females in the present no-delay condition. Further, a

novel environment also increases plasma levels of ACTH and corticosterone to higher levels for a longer period of time in female rats as opposed to males (Brett et al. 1983, Kitay 1961). Thus, the compound effects of high levels of stress hormone release in response to both MK801 and novelty could be manifest as the high level and extended duration of locomotor activity in female rats in the delay condition.

The present study demonstrates the first appearance of the gender differences in locomotor responses to MK801 in 30-day-old pups. This time of ontological onset supports a role for gonadal hormones in modulating the response to MK801 because this developmental stage approaches "periadolescence" in the rat pup, i.e. the initiation of sex hormone circulation in young rats and the stage of vaginal opening for females (Losada et al. 1993, Spear and Brake 1983).

Central drug effects are not likely to mediate the gender differences in behavioral response to MK801. Nevertheless, there are a few central locations in which gender differences in neural response to MK801 and the novel environment might be possible. Female gonadal hormones can directly modulate the binding of MK801, NMDA and glutamate in the hippocampus (Weiland 1992) and can modify behaviors elicited by the striatum (Joyce and Van Hartesveldt 1984), perhaps including locomotor responses to novelty. The striking lack of habituation to the test monitor in adult females in the delay conditions would support a gender-dependent difference in hippocampal or amygdala function, given that these regions, as opposed to cortical or ventral striatal regions, have been implicated in the continuation of motor activity beyond initial approach movements in the presence of a novel food (Burns et al. 1996). Also along these lines, the similarity in the delay-potentiated activity of male and female rats may indicate that MK801 has

similar function in the medial prefrontal cortex in males and females, because this region may mediate initial reactions to novelty (Burns et al. 1996).

There are several main findings of this study. 1) Environmental novelty and MK801 interact to produce higher levels of locomotion than either stimulus alone, in rats 20 days of age and older. The effect may be mediated by an interaction between stress hormones and MK801. 2) The locomotor activating effects of MK801 are lowest in 10-day-old rat pups and highest in 20-day-olds, mimicking age-related changes in novelty-induced locomotion. These ontological changes may reflect maturation of limbic-motor circuitry. 3) Thirty-day-old and adult female rats are more sensitive to the motor effects of MK801. This effect is most likely attributable to gender differences in MK801 metabolism.

Research on MK801-induced locomotion has clinical implications for schizophrenia and Parkinson's disease, two human conditions in which abnormal interactions between glutamate and dopamine in mesocorticolimbic and basal ganglia circuitry produce cognitive, emotional and motor dysfunction. While dopamine has long been a target for pharmacological treatment of these disorders, glutamate has become a new target for pharmacotherapies (Carlsson et al. 1997, Carlsson and Carlsson 1990, Halberstadt 1995, Moghaddam 1994, Olney and Farber 1995a and b, Wachtel and Turski 1990). The gender-dependent effects of MK801 caution against the assumption that glutamatergic drugs will be equally effective in male and female patients, especially if females are in simultaneous estrogen replacement therapy. The analysis of ontological changes in novelty- and MK801-induced locomotion may define normal limbic-motor

maturation against which abnormal development, as in the case of schizophrenia, can be compared.

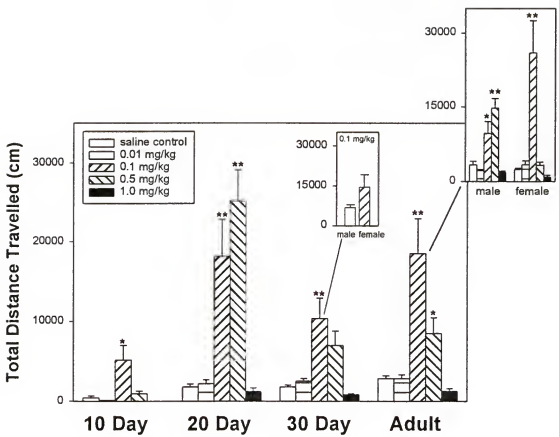

Figure 2-1. MK801-induced locomotion in a two-hour test session.

Total distance (cm) travelled in a 2 h test session by rats of several different ages following subcutaneous injection with various doses of MK801. Significant differences from saline-injected controls are indicated (**P<0.01, *P<0.05). Error bars represent standard errors of the mean. Insets show data for males and females separately.

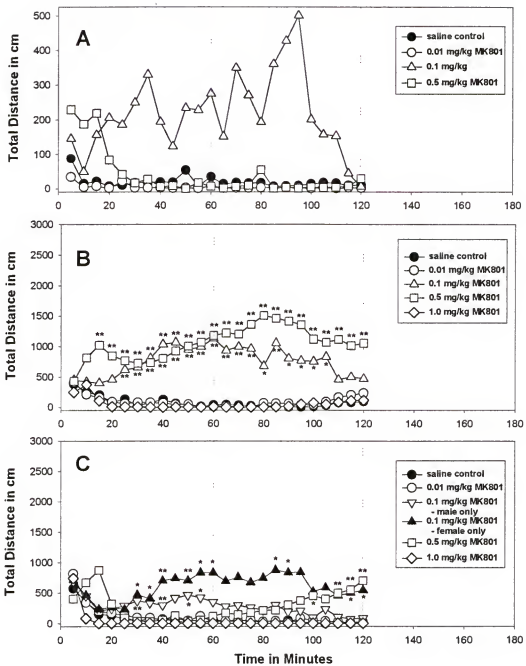

Figure 2-2. Time course of MK801-induced locomotion in rat pups.

A) Distance travelled (cm) by rat pups at 10 days of age following subcutaneous injection of various doses of MK801 and placement into activity monitors immediately after injection; B) The same measure for 20-day-old pups; C) The same measure for 30-day-old pups. Significant differences from saline-injected control groups are shown (**P<0.01, *P<0.05). Note the difference in range of the ordinate on graph A compared with graphs B and C. Data for male and female rats are combined for all groups except 30-day-old rats given 0.1 mg/kg MK801 (C). Error bars have been omitted for clarity.

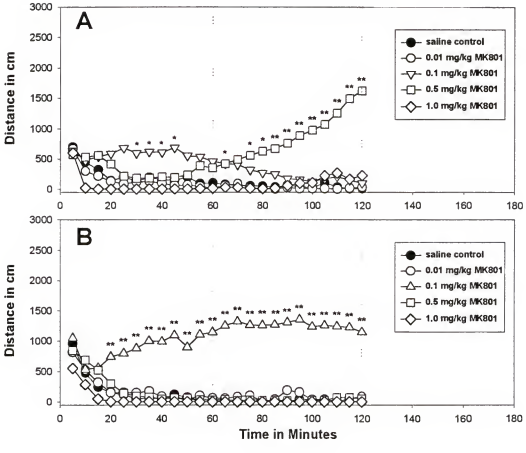

Figure 2-3. Time course of MK801-induced locomotion in adult rats.

A) Distance travelled (cm) by adult male rats following subcutaneous injection of various doses of MK801 saline and placement into activity monitors immediately after injection; B) The same measure for adult female rats. Significant differences from saline-injected control groups are shown (**P<0.01, *P<0.05).

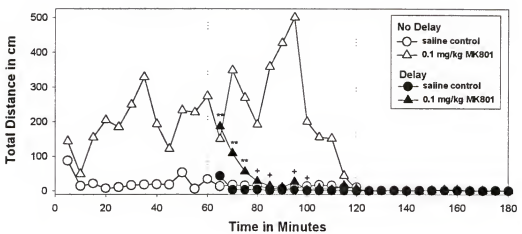

Figure 2-4. Distance travelled by ten-day-old rat pups: no-delay vs. delay.

Distance travelled (cm) by 10-day-old rat pups following subcutaneous injection of 0.1 mg/kg MK801 or saline and placement into activity monitors either immediately after injection (data start at 5 min mark) or after a 60 min delay (data start at 65 min mark). Significant differences from control groups under the same timing conditions are shown (**$P<0.01$), as are differences between no-delay and delay groups (+$P<0.05$).

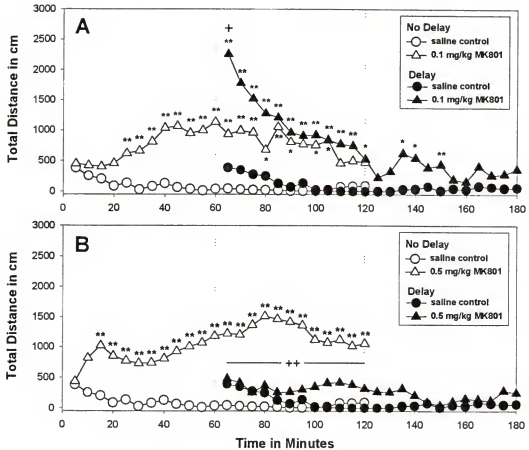

Figure 2-5. Distance travelled by twenty-day-old rat pups: no-delay vs. delay.

A) Distance travelled (cm) by 20-day-old rat pups following subcutaneous injection of 0.1mg/kg MK801 or saline and placement into activity monitors either immediately after injection (data start at 5 min mark) or after a 60 min delay (data start at 65 min mark); B) The same measure following 0.5mg/kg MK801 or saline. Significant differences from control groups under the same timing conditions are shown (**P<0.01, *P<0.05), as are differences between no-delay and delay groups (++P<0.01, +P<0.05).

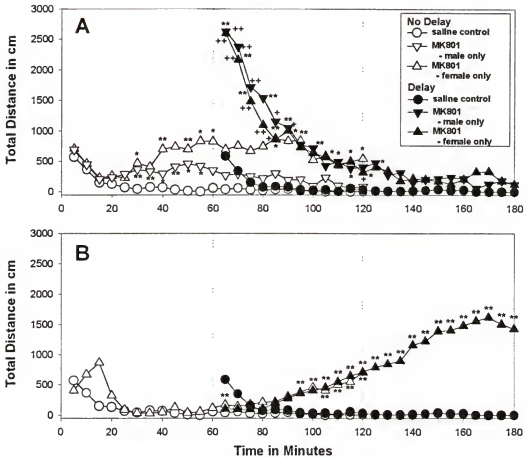

Figure 2-6. Distance travelled by thirty-day-old rat pups: no-delay vs. delay.

A) Distance travelled (cm) by rat pups at 30 days of age following subcutaneous injection of 0.1mg/kg MK801 or saline and placement into activity monitors either immediately after injection (data start at 5 min mark) or after a 60 min delay (data start at 65 min mark); B) The same measure following 0.5mg/kg MK801 or saline. Significant differences from control groups under the same timing conditions are shown (**P<0.01, *P<0.05), as are differences between no-delay and delay groups (++P<0.01, +P<0.05). Male and female groups are shown separately in A but are combined in B.

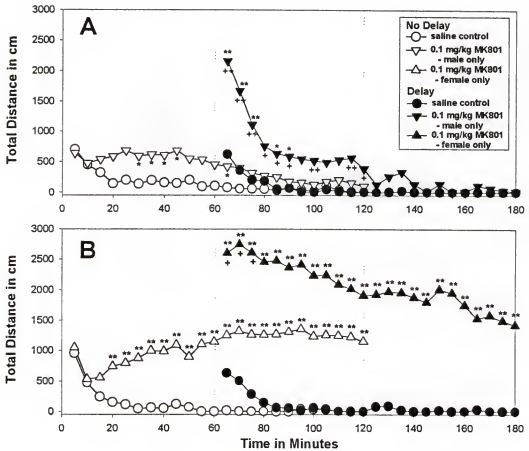

Figure 2-7. Distance travelled by adult rats given 0.1mg/kg MK801: no-delay vs. delay. A) Distance travelled (cm) by adult male rats following subcutaneous injection of 0.1 mg/kg MK801 or saline and placement into activity monitors either immediately after injection (data start at 5 min mark) or after a 60 min delay (data start at 65 min mark); B) The same measure for female rats. Significant differences from control groups under the same timing conditions are shown (**P<0.01, *P<0.05), as are differences between no-delay and delay groups (++P<0.01, +P<0.05).

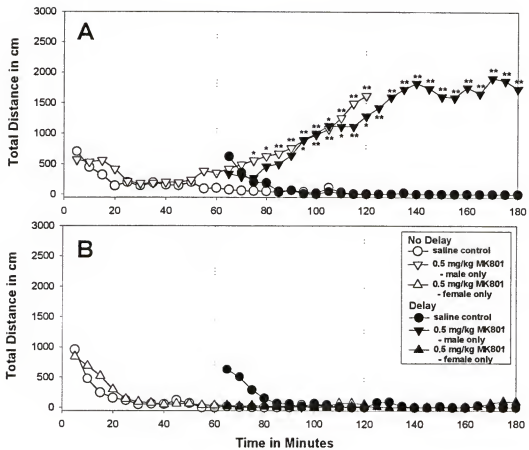

Fig. 2-8. Distance travelled by adult rats given 0.5mg/kg MK801: no-delay vs. delay.

A) Distance travelled (cm) by adult male rats following subcutaneous injection of 0.5 mg/kg MK801 or saline and placement into activity monitors either immediately after injection (data start at 5 min mark) or after a 60 min delay (data start at 65 min mark); B) The same measure for female rats. Significant differences from control groups under the same timing conditions are shown (**P<0.01, *P<0.05).

CHAPTER 3

THE LOCOMOTOR EFFECTS OF MK801 IN THE NUCLEUS ACCUMBENS OF DEVELOPING AND ADULT RATS

Introduction

The nucleus accumbens is a major site of limbic-motor interface in the brain due to its integral role in locomotion, reward-related behaviors and responses to stress (Mogenson et al. 1993). The excitatory amino acid, glutamate, is projected into nucleus accumbens from the limbic neocortex, amygdala and hippocampus and the classic motor stimulant, dopamine, is projected from midbrain motor nuclei of the ventral tegmental area into the nucleus accumbens (Heimer et al. 1993, for review). Whereas dopamine has long been recognized as a major modulator of locomotion, reward-related behaviors and responses to stress (LeMoal and Simon 1991, Salamone 1994, 1996, for reviews), an important role for glutamate in such behaviors has only recently been confirmed (Burns et al. 1994, Carlezon and Wise 1996, Horger and Roth 1995, Kelley and Throne 1992, Moghaddam 1993, Willner et al. 1992). The behavioral function of glutamate deserves further investigation.

Behavioral studies of the adult rat demonstrate the major role of nucleus accumbens glutamate in locomotion. Locomotor behavior can result from direct intra-accumbens injections of either agonists or antagonists at the *N*-methyl-D-aspartate (NMDA) receptor subtype (e.g. Al-Khatib et al. 1995, Boldry and Uretsky 1988, Burns et

al. 1994, Donzanti and Uretsky 1988, Hamilton et al. 1986, Svensson et al. 1994, Svensson and Carlsson 1992, Wu et al. 1993b) or other glutamate receptor subtypes (e.g. Boldry et al. 1991, Burns et al. 1994, Hamilton et al. 1986, Wu et al. 1993b). Systemically-administered NMDA receptor antagonists, such as MK801 (dizocilpine), produce an inverse U-shaped dose-response curve with respect to locomotion (Carlsson and Carlsson 1989, Willins et al. 1993, Ford et al. 1989, Hargreaves and Cain 1992) and the stimulatory aspects of the response can be eliminated by pretreatment of the nucleus accumbens with antagonists at other glutamate receptor subtypes (Willins et al. 1993). Furthermore, classic motor stimulants, such as peripherally-injected cocaine (Pulvirenti et al. 1991) or intra-accumbens-injected amphetamine (Burns et al. 1994, Kelley and Throne 1992) or dopamine (Hamilton et al. 1986) produce locomotion that can also be blocked by intra-accumbens injection of an NMDA receptor antagonist. The locomotor effects of glutamate in the nucleus accumbens are obviously complex, given that both agonists and antagonists of glutamate receptors can increase locomotion and that the activity they elicit can be blocked by other glutamatergic drugs (Hamilton et al. 1986). The role of glutamate in locomotion may be determined in part by interactions with dopamine or by the novelty of a testing situation.

In fact, the locomotor effects of glutamatergic drugs depend in part on dopamine transmission in the nucleus accumbens. Intra-accumbens injection of dopamine receptor antagonists can block the stimulatory effects of peripherally-injected MK801 (Ouagazzal et al. 1994, Willins et al. 1993). When dopaminergic and glutamatergic ligands are both administered to the nucleus accumbens, dopamine agonists can even block the activity induced by NMDA itself (Boldry and Uretsky 1988, Wu et al. 1993b). In animals

depleted of nucleus accumbens dopamine by reserpine pretreatment or 6-hydroxy-dopamine lesions of dopamine terminals, MK801 induces less locomotion than in rats with intact dopamine function (Carlsson and Carlsson 1989, Carlsson and Svensson 1990, Criswell et al. 1993, Ferré et al. 1994, Ouagazzal et al. 1994, Starr and Starr 1993 and 1994, Svensson et al. 1992 a and b, 1994, Svensson and Carlsson 1992). Peripherally-injected drug combinations also confirm that dopamine and glutamate interact to alter locomotion (e.g. Dall'Olio et al. 1996, Hoffman 1992, Ögren and Goldstein 1994).

The locomotor effects of both glutamate and dopamine in the nucleus accumbens depend in part on the novelty of the external environment. Intra-accumbens injection of a glutamate antagonist (Hooks et al. 1992, Bradberry et al. 1991, Mogenson and Nielson 1984) or a dopamine agonist (Mogenson and Wu 1991a and b) decreases locomotion that is induced by the presence of novel objects. However, intra-accumbens injection of a glutamate antagonist increases locomotor activity in animals habituated to their environment (e.g. Al-Khatib et al. 1995, Svensson et al. 1994) or exposed to a novel environment that does not contain novel objects (e.g. Boldry and Uretsky 1988, Maldonado-Irizarry and Kelley 1994). Likewise, intra-accumbens injection of a dopamine agonist increases locomotion in animals habituated to their environments (Wu et al. 1993a). Novelty in the environment can completely reverse the direction of a drug response to dopamine agonists injected peripherally (Van Hartesveldt 1997) or into the nucleus accumbens (Wu et al. 1993a), but the neural mechanisms underlying the effect are unclear. Experiments in which locomotion induced by environmental novelty is altered by lesions of the medial prefrontal cortex, hippocampus or amygdala, the major

sources of glutamate input to the nucleus accumbens (Braun et al. 1993, Burns et al. 1996, Jellestad and Bakke 1985, Lacroix et al. 1998, Lipska et al. 1992, 1994, Lorenzini et al. 1991, Means et al. 1971), give some indication of the neuroanatomical substrates involved.

Notably, novelty influences locomotion in an age-dependent manner. Locomotor exploration of a novel environment is barely exhibited by 10-day-old rats but the behavior rises rapidly to a peak in 15-day-old rat pups, only to fall again to adult levels by 25 days of age (Barrett et al. 1982, Campbell et al. 1969, Spear and Brake 1983). Furthermore, while intra-accumbens or peripheral injections of dopamine agonists can suppress locomotion induced by novelty in older rat pups and adults (Van Hartesveldt et al. 1992, 1994, Mogenson and Wu 1991a, b), novelty-induced locomotion is not suppressed by intra-accumbens injections of quinpirole in rat pups 11 days of age (Frantz and Van Hartesveldt 1995). In general, novelty-dependent, dopamine agonist-induced locomotor suppression occurs only in rat pups older than approximately three weeks of age and does not reach adult levels until after postnatal day 30 (Arnt 1983, Frantz et al. 1996, Shalaby et al. 1981, Spear and Brake 1983). The late ontological onset of the interaction between novelty and dopaminergic compounds indicates that it requires a neural mechanism that does not mature until approximately the third postnatal week.

The afferent glutamate projections to the nucleus accumbens could provide the mechanism through which novelty modulates nucleus accumbens transmitter activity. The sources of glutamate projections, the limbic cortex, amygdala and hippocampus all play a role in responding to novelty and stress (Abercrombie et al. 1989, Bradberry et al 1991, Burns et al. 1996, Feenstra et al. 1995, Hooks and Kalivas 1994, Horger and Roth

1995, Moghaddam 1993, Rebec et al. 1997). The prefrontal cortex and hippocampus mature relatively late in ontogeny (Angevine, Jr. 1975, Alexander and Goldman 1978, Benes 1989, O'Keefe and Nadel 1978), making their glutamatergic projections into the nucleus accumbens likely candidates for late-maturing modifiers of dopaminergic response.

Further studies should be conducted to clarify the mechanisms of these phenomena. Toward that end, and with the independent aim of exploring ontological changes in glutamate transmission, the role of glutamate itself in locomotion across ontogeny should be better defined. Glutamate activity at NMDA glutamate receptors can influence locomotion in developing rats; subcutaneous injections of the NMDA receptor antagonist, MK801, produce an adult-like inverse U-shaped dose-response curve with regard to locomotor activity in rats across ontogeny (Rajachandran et al. 1991, Scalzo and Burge 1994, also see Chapter 2). Whereas 0.1 or 0.2 mg/kg doses of MK801 increase motor behavior, 0.5 or 1.0 mg/kg doses produce ataxia in pups as young as 3-4 days old. In pups 12 days of age and younger, the motor activation is sporadic and low in magnitude. In older pups and adults, the coordinated motor behaviors produced by lower doses include forward locomotion, sniffing, turning and rearing, whereas the ataxia produced by higher doses at all ages includes body rolling, akinesia, a lack of limb coordination and a loss of postural support. In adult male rats, locomotor activation can be elicited by injection of MK801 directly into the nucleus accumbens (Al-Khatib et al. 1995). Thirty-day-old and adult female rats are more sensitive than males to the locomotor effects of peripherally-injected MK801 (e.g. Blanchard et al. 1992, Criswell et al. 1993, Fleischmann et al. 1991, Haggerty and Brown 1996, Hönack and Löscher et al.

1993, also see Chapter 2). Yet, these peripheral injections provide little information as to the anatomical site of drug action.

The aim of the present study was to explore the locomotor effects of glutamate activity at NMDA receptors specifically in the nucleus accumbens across ontogeny. The non-competitive NMDA receptor antagonist, MK801, was employed in this study because MK801 is a use-dependent, open-channel blocker of the NMDA receptor-associated ion channel. Therefore, its effects on locomotion indicate that endogenous glutamate had already activated the NMDA receptor and opened the associated ionophore. By injecting MK801 directly into the nucleus accumbens of both male and female, developing and adult rats, and analyzing their subsequent locomotor responses, the role of nucleus accumbens glutamate activity in the age- and gender-dependent locomotor effects of MK801 was investigated.

Materials and Methods

Subjects

Rats were bred and housed as described in Chapter 1. Rat pups were implanted with injection cannulae at 10, 20 and 30 days of age and tested the next day at 11, 21, and 31 days of age. Adult rats were between 60 and 65 days of age at the time of cannulae implantation. Each dose group consisted of 6 to 15 rats with approximately equal numbers of males and females in each group. Rats from 2 to 5 different litters were tested at each drug dose. Each rat was tested only once.

Surgery

Stereotaxic surgery was carried out while 10-day-old rat pups were under hypothermic anesthesia and older pups and adults were under ketamine/xylazine

anesthesia. Metofane was used to supplement the primary anesthesia, if necessary. Guide cannulae constructed from 23 ga stainless steel tubing were implanted bilaterally above the nucleus accumbens, using coordinates determined empirically for each age group.

In order to analyze the specificity of the intra-accumbens drug placement, the injection site was varied along the dorsal-ventral plane by using injection needles of several different lengths to inject 10 μg MK801 into the brains of 21- and 31-day-old rat pups. Eleven-day-old pups were not used in the placement analysis because pilot experiments revealed no profound locomotor responding to the drug placed at several cerebral locations. Nor were adult rats used in the placement analysis because they exhibited significant locomotion only in response to higher doses of intra-accumbens MK801. These higher doses were injected in higher volumes, tended to diffuse more, and consequently obscured analysis of placement specificity. Instead, 4 adult rats were implanted with cannulae above the lateral ventricles to enable intra-cerebroventricular injection of MK801.

All rats were allowed approximately 24 h to recover from surgery. Rat pups were returned to their pre-surgery housing conditions for the interval between surgery and testing. Adult rats, which had previously been group-housed, were isolated for the interval between surgery and testing in order to protect the implanted cannulae.

Drug Procedure

(+)MK-801 hydrogen maleate (dizocilpine maleate, Research Biochemicals International, MA, USA), was dissolved over heat in glacial acetic acid, then pH-

balanced with 1N NaOH to a pH of approximately 4.85. The solution was either used fresh or frozen in aliquots for use on the day of testing. MK801 was injected bilaterally into the nucleus accumbens at doses of 0 μg (dH_2O control injection), 3, 10, 20 or 40 μg). The insolubility of the drug mandated that higher volumes be injected when higher doses of the drug were administered, after the lower dose groups had been tested. Thus, MK801 was injected in volumes of 0.25 μl (0, 3 and 10 μg doses), 0.50 μl (20 μg dose) and 1.00 μl (40 μg dose). The solutions were equivalent to 0, 0.039 or 0.12 M concentrations. Because the MK801 solution was acidic, a separate control group of rats was injected with 0.5 μl of glacial acetic acid/1 N NaOH solution with a pH of approximately 4.85. Testing this group enabled analysis of the locomotor and histological effects of acid injection.

Behavioral Procedure: Intra-Accumbens MK801 Injections

On the day of testing, rats were transported in their home cages from the colony rooms to the working laboratory. Eleven- and 21-day-old rats were maintained with their dams until just before drug injection. Drug injection took place in the testing room. The 30 ga injection needles were inserted through the guide cannulae into the target region and the vehicle or drug was infused at a rate of 0.50 μl per min. The injection needles were left in place for an additional 30 sec before removal. Immediately thereafter, each rat was placed in an activity monitor which recorded locomotion for 2 h, as described in Chapter 1. Experimenter observation of locomotor behavior took place simultaneously with the automated measure of locomotion, in order to gauge qualitatively the MK801-induced motor syndrome.

Behavioral Procedure: Effects of Ketamine/Xylazine Pretreatment on MK-801-Induced Locomotion

The most effective anesthesia for rat pups and adult rats in our laboratories is a drug combination of 5 parts ketamine to 1 part xylazine. Ketamine, like MK801, is a non-competitive, glutamate NMDA receptor antagonist that binds to the same receptor site as MK801. Thus, it is feasible that treatment with the ketamine/xylazine cocktail on the day of surgery affected locomotor responses to MK801 approximately 24 h later.

In order to investigate this possibility, rat pups of 20 and 30 days of age were injected with the anesthetic dose of the ketamine/xylazine cocktail and were allowed to recover without undergoing surgery. Approximately 24 h later, they were injected subcutaneously with various doses of MK801 (0.00, 0.01, 0.1, 0.5, or 1.0 mg/kg) in a volume of 2.5 ml/kg. Immediately after drug injection, they were placed in the activity monitors for a 2 h test session. Results were compared with findings from Chapter 1 in the no delay condition in which subcutaneous MK801 injection was followed immediately by placement of the rat in the activity monitor.

Histology

After behavioral testing of intracerebral drug injections, rats were administered an overdose of sodium pentobarbital (i.p.) and perfused intracardially with 0.9% NaCl followed by 10% formalin. The brains were removed and placed in a 10% sucrose-10% formalin solution. At least 24 h later, the brains were frozen, sectioned, mounted on slides and stained with thionin. The locations of the injection needle tips were identified, and only animals with appropriately placed bilateral injections were used in the analysis. After intra-cerebroventricular drug injections, rats were administered ketamine/xylazine

anesthesia (i.p.) and were decapitated. ICV injection was verified by injecting dye through the cannulae and observing dye ejection from the caudal aspect of the brain.

Statistics

The total distance travelled in cm was analyzed in all the conditions tested. Separate three-way analyses of variance with repeated measures (time factor) were carried out for each age group with drug condition, gender and time interval as the main factors. Follow-up comparisons were made using two-way and one-way ANOVAs followed by Duncan's New Multiple Range Test to determine significance at the $P < 0.05$ or $P < 0.01$ level. One-way ANOVAs were also conducted to compare the total distance of activity induced by each drug dose throughout the entire 2 h test session. There were no significant differences between males and females in response to intracerebral drug injections. Therefore, males and females were analyzed as one group. In the analysis of ketamine/xylazine pretreatment effects, male and female 31-day-old pups were analyzed separately.

The behavior of 11-day-old rat pups was best represented by a measure of repetitive interruption of the same photocell beam in the activity monitors. These 'stereotypy counts' were analyzed, in addition to the total distance travelled (cm). Even the stereotypy counts of 11-day-old rat pups were highly inconsistent across the group, though, so pups were classified as responders or non-responders to the locomotor-activating effects of MK801. Responders had z-scores greater than 1.96, i.e. their activity levels were outside the 95% confidence interval for the mean of the dH_2O-injected group. The stereotypy counts of only the subset of 11-day-old responders were analyzed in 15 min intervals over the 2 h test session.

Results

Injections Targeted at the Nucleus Accumbens

When injections of MK801 were targeted bilaterally toward the nucleus accumbens of developing and adult rats, the locomotor responses depended on the dose of the drug and the age of the rat (Fig. 3-1). A subset of 11-day-old rat pups exhibited sporadic bouts of locomotor activity that resembled obstinate progression (head pressed into a corner but limbs continuing to step in a coordinated movement pattern) in response to 3 and 10 μg doses of MK801 (Fig. 3-2). Other 11-day-olds did not exhibit significant changes in behavior in comparison with dH_2O-injected controls. Twenty-one-day-old pups responded to mid-range doses of MK801 with a high magnitude of locomotor activation and even exhibited ataxia in response a high dose of MK801 (Fig. 3-3). Thirty-one-day-old pups and adults exhibited robust locomotor activation to the mid-range and high doses of MK801, but the high doses, injected in high volumes, may have spread into the lateral ventricles (Fig. 3-4 and 3-5). Generally, the locomotor activation consisted of hyperlocomotion, stereotyped sniffing, "frantic exploration" of the test monitor, hyper-reactivity and hyper-excitability. The ataxia in 21-day-olds consisted of head weaving, reciprocal forepaw treading, body rolling and decreased rearing. It progressed into a debilitating ataxia, involving a flattened posture, footsplay, immobility, akinesia, salivation and lacrimation. The quality of activity resembled that described by Sturgeon et al. (1979) who designed a rating scale for locomotor activity, stereotypy and ataxia induced by phencyclidine, a compound that binds to the same pharmacological site as MK801 and exerts similar behavioral effects. These behaviors were in stark contrast to

those of dH_2O-injected control rats which explored the novel environment initially but habituated within approximately 20 min.

Eleven-day-old rat pups

Intra-accumbens MK801 (3 or 10 μg) elicited infrequent, short, sporadic bouts of motor activity in 37% of the 11-day-old rat pups. This response consisted mainly of forepaw treading and attempted rearing in the perimeter of the test arena. It resembled obstinate progression, and was sometimes followed by wall-climbing. The pups not exhibiting the bouts of motor activity did not exhibit any other noticeable behaviors different from the dH_2O-injected control group. Thus, with all the subjects included in a statistical analysis, MK801 did not significantly alter either the total stereotypy counts [$F(2,22)=4.06$](data not shown) or the total distance travelled in cm during the entire 2 h test session [$F(2,22)=2.10$] (Fig. 3-1).

Further analyses were carried out with only those rat pups that exhibited the motor response. The behavior is best represented by the stereotypy counts recorded by the activity monitors (Fig. 3-2). Responders were defined as rats with z-scores greater than 1.96 (see methods). This criterion left 4 pups in the 3 μg dose-group and 3 pups in the 10 μg dose-group, to be compared with the 6 pups in the control group. Of the 7 rats responding to MK801, 5 were female, a non-significant difference in proportions between males and females exhibiting the response, according to a non-parametric test for significant differences in proportions (Bruning and Kintz 1968). Each individual pup exhibited bouts of activity that lasted 5-15 min, as shown in Fig. 3-2B for two representative subjects. For the group, 3 μg MK801 increased activity above control

levels at the 90 and 120 min intervals ($P<0.05$); 10 μg MK801 increased activity at the 15 min interval ($P<0.05$). These differences in stereotypy counts were confirmed by significant dose effects [$F(2,10)=18.857$, $P<0.0004$] and a significant dose x time interaction [$F(14,70)=1.86$, $P<0.0459$] (Fig. 3-2A). Higher doses of MK801 were not administered to rat pups at this age because a slight decline in motor activity induced by 10 μg compared with 3 μg indicated that higher doses may induce severe ataxia or may be toxic to the animal.

Histological analysis of the brain tissue was used to insure that only rats with injections aimed into the nucleus accumbens were included in the analysis. The injection site was just lateral to the anterior commissure (Figs. 3-2 inset and 3-6A).

Twenty-one-day-old rat pups

In 21-day-old rat pups, centrally-injected MK801 (3, 10, 20 μg) produced an inverse U-shaped dose-effect curve with respect to the total distance travelled in cm over the 2 h test session (Fig. 3-1). The 3 μg dose did not significantly change locomotion; the 10 μg dose substantially increased the total distance travelled, and 20 μg MK801 induced ataxia to the extent that the pups were immobile. Their activity levels were comparable to those of dH_2O-injected control rats. No significant effects of gender were recorded.

The total distance travelled in 5 min intervals was different according to significant dose [$F(3,34)=15.74$, $P<0.0001$] and time [$F(23,782)=1.85$, $P<0.01$] effects and a dose x time interaction [$F(69,782)=1.86$, $P<0.0001$] (Fig. 3-3). The 10 μg dose consistently increased the distance travelled from 15 min post-injection through the end of the test session ($P<0.01$ at 15-70 min and 85-120 min intervals, $P<0.05$ at 75-90 min

intervals), compared with dH_2O-injected rats. Rats injected with the 3 μg or 20 μg doses did not exhibit significantly different activity levels.

Histological analyses showed that the injections were placed in the nucleus accumbens, just medial to the anterior commissure (Figs. 3-3 inset and 3-6B). The higher injection volume of 0.5μl for the 20 μg dose of MK801 (compared with 0.25μl for the other doses) left a visible marking of solution diffusion (Fig. 3-7A). In adult rat brain, such tissue alteration was caused by the acidic nature of the solution (pH: 4.85), as demonstrated when control injections of acidic solution not containing MK801 caused the same lack of staining, but dH_2O injections did not.

Thirty-one-day-old rat pups

In 31-day-old rat pups, centrally-injected MK801 (3, 10, 20, 40 μg) altered the total distance travelled in cm over the entire 2 h test session in a dose-dependent manner (Fig. 3-1). The 3 μg dose did not significantly alter locomotor activity, but the 10, 20 and 40 μg doses substantially increased the distance travelled [$F(4,49)=29.67$, $P<0.0001$]. No significant effects of gender were recorded.

The total distance travelled in 5 min intervals was significantly different according to significant dose [$F(4,49)=29.665$, $P<0.0001$] and time [$F(23,1127)=4.662$, $P<0.0001$] effects and a significant dose x time interaction [$F(92,1127)=5.88$, $P<0.0001$] (Fig. 3-4). The 10 μg dose increased the distance travelled from approximately 25-75 min post-injection ($P<0.01$ at 30, 40 and 45 min intervals, $P<0.05$ at the 25, 35, 50, 55 and 75 min intervals), compared with vehicle-injected rats. The 20 μg dose increased the distance travelled more consistently from 20 min post-injection through the end of the

test session ($P<0.01$). Similarly, 40 μg MK801 increased the distance travelled from 25 min post-injection through the end of the session ($P<0.01$ at 30, 45 and 55-120 min intervals; $P<0.05$ at 25, 35 and 50 min intervals). Rats injected with the 3 μg dose did not exhibit significantly different activity levels.

Histological analyses showed that the injections of dH_2O, 3 μg and 10 μg MK801 were placed in the nucleus accumbens, just medial to the anterior commissure (Figs. -4 inset and 3-6C). The higher injection volumes of 0.5 μl for the 20 μg dose and 1.0μl for the 40 μg dose of MK801 (compared with 0.25 μl for other doses) left a visible marking of solution diffusion (Fig. 3-7B). This marking was determined to be caused by the acidic nature of the solution (pH=4.85). In addition, it appears that the 20 and 40 μg doses of MK801 may have spread dorsal into the lateral ventricles instead of being localized in the nucleus accumbens and, in some cases, they diffused more than 1 mm in the rostral-caudal dimension.

Adult rats

In adult rats, centrally-injected MK801 (3, 10, 20, 40 μg) altered the total distance travelled in cm over the entire 2 h test session in a dose-dependent manner (Fig. 3-1). Neither the 3 nor the 10 μg dose altered locomotor activity, but the 20 and 40 μg doses substantially increased the distance travelled. No significant gender effects were found.

The total distance travelled in 5 min intervals was significantly different according to significant dose [$F(4,51)=5.2321$, $P<0.01$] and time [$F(23,1173)=4.3143$, $P<0.0001$] effects and a dose x time interaction [$F(92,1173)=5.08$, $P<0.0001$] (Fig. 3-5). The 20 μg dose increased the distance travelled from approximately 30 min post-injection

almost to the end of the session ($P<0.01$ at 40-50, and 70 min intervals, $P<0.05$ at 30, 55-65 and 75-115 min intervals), compared with dH_2O-injected rats. The 40 μg dose increased the distance travelled with a longer latency to the beginning of the increase; it started more than 1 h post-injection, but then continued through the end of the session ($P<0.01$ at 85-120 min intervals, $P<0.05$ at 70-80 min intervals). Rats injected with the 3 μg and 10 μg doses did not exhibit significantly different activity levels.

Histological analyses showed that the injections of dH_2O, 3 μg and 10 μg MK801 were placed in the nucleus accumbens, just medial to the anterior commissure (Figs. 3-5 inset and 3-6D). However, the higher injection volumes of 0.5μl for the 20 μg dose and 1.0μl for the 40 μg dose of MK801 (compared with 0.25μl for the other doses) left a visible marker of solution diffusion, caused by the acidic nature of the MK801 solution (pH: 4.85, Fig. 3-7C).

In order to investigate whether this apparent damage to the brain tissue would cause a motor response itself, 7 rats (54-56 days of age) were injected with 0.5μl of a glacial acetic acid/ NaOH solution with pH = 4.85 and their locomotor activity was recorded. A one-way ANOVA and Duncan's Follow-Up analysis showed that the distance travelled by the acid-injected control group did not differ from the dH_2O-injected controls, but both differed significantly from the 20 μg MK801-injected experimental group [$F(2,30)=10.89$, $P<0.0003$] (Fig. 3-8).

In addition, as in the 31-day-old pups, it appears that the 20 and 40 μg doses may have spread into the lateral ventricles instead of being localized near the nucleus

accumbens of adult rats. Thus, specific intracerebroventricular injections of MK801 were administered for comparison, as described below.

Examination of Placement Specificity

Twenty-one- and 31-day-old rat pups

In order to investigate the possibility that the MK801 solution diffused back up the cannula track to produce its locomotor effects from a location dorsal to the nucleus accumbens, the locomotor effects of 10 μg MK801 injected dorsal to the accumbens were recorded. Twenty-one- and 31-day-old rat pups were used in this experiment because they exhibited increased locomotion in response to intra-accumbens injection of 10 μg MK801 in the volume of 0.25μl, which did not observably spread into the cerebral ventricles. Neither 11-day-old nor adult rats were used in these conditions (see methods for explanation).

The injection site was a larger determinant of locomotor responding in 31-day-old than in 21-day-old rat pups (Fig. 3-9A and B). In 31-day-old rat pups, 10 μg MK801 in a mid-striatal placement failed to increase locomotion to the extent that intra-accumbens MK801 did, whereas a mid-striatal injection placement in 21-day-old rats produced the same increase in locomotion as intra-accumbens injections. Placements in the dorsal striatum (21- and 31-day-old pups) or near the corpus callosum (31-day-old pups only) failed to increase locomotion significantly above the levels of dH_2O-injected rats at either age. Such striatal placements of MK801 did not induce profound levels of other stereotypies to the extent that they might have interfered with the expression of locomotion, as determined by experimenter observation.

In 21-day-old rat pups, statistical tests confirmed the significance of these findings such that a two-way ANOVA showed significant placement [$F(2,26)=11.386$, $P<0.0003$] and time [$F(23,598)=5.229$, $P<0.0001$] effects and a significant placement x time interaction [$F(46.598)=2.01$, $P<0.0001$] (Fig. 3-9A). The mid-striatal injection of MK801 induced activity that differed from the intra-accumbens injection group at only 4 time intervals in the test session ($P<0.01$ at 80 min; $P<0.05$ at 100, 105 and 110 min). On the other hand, the dorsal striatal injection induced significantly less activity than the intra-accumbens injection throughout most of the test session ($P<0.01$ at 15, 20, 55, 65, 80 and 95-120 min intervals; $P<0.05$ at 25, 60, 70, 85 and 90 min).

In 31-day-old rat pups, the two-way ANOVA showed a significant placement [$F(3,32)=7.023$, $P<0.0009$] and time [$F(23,736)=13.894$, $P<0.0001$] effects and a significant placement x time interaction [$F(69,736)=2.16$, $P<0.0001$] (Fig. 3-9B). In all of the striatal placements, MK801 elicited activity levels that were significantly lower than the intra-accumbens injection group from approximately 25-90 min post-injection.

Intracerebroventricular injections to adult rats

In order to investigate the possibility that the 20 and 40 μg doses of MK801 diffused into the lateral ventricles and produced their locomotor effects by distributing to various circumventricular loci, 20 μg MK801 was injected directly into the lateral ventricles and the locomotor responses were compared with effects of injections aimed toward the nucleus accumbens.

Intracerebroventricular (ICV) injections of 20 μg MK801 to adult rats increased locomotor activity with a shorter latency than injections aimed toward the nucleus

accumbens (Fig. 3-10). A two-way ANOVA revealed significant effects of drug condition [$F(2,26)=5.8432$, $P<0.008$] and time [$F(23,598)=1.7664$, $P<0.0154$] as well as a significant drug condition x time interaction [$F(46,598)=5.1853$, $P<0.0001$]. During the first twenty minutes of the test session, the ICV injections significantly increased the distance travelled, relative to both the dH_2O-injected control group ($P<0.01$ at the 5 min interval; $P<0.05$ at the 10, 15 and 20 min intervals) and the intra-accumbens MK801 group ($P<0.01$ at the 5, 15 and 20 min intervals; $P<0.05$ at the 10 min interval). In contrast, the intra-accumbens MK801 did not significantly increase the distance travelled above control levels until 25 min post-injection, but the activity then remained significantly increased through the end of the session ($P<0.01$ at the 40, 55 and 70 min intervals; $P<0.05$ at the 25-35, 45, 50, 60, 65, 75-105, 115 and 120 min intervals). The small group size (n=4) of the ICV injection group may have weakened the power of the statistical tests, resulting in mean activity levels that did not differ significantly from those of the control group.

Effects of Ketamine/Xylazine Pretreatment on MK801-Induced Locomotion

The ketamine solution used as anesthesia for the guide cannula implantation surgery binds to the PCP site inside the NMDA receptor-associated ion channel, as does MK801. Therefore, the possibility that ketamine pretreatment 24 h before MK801 administration might alter the subsequent locomotor response to MK801 was investigated by administering ketamine to 21- and 31-day-old rat pups and recording their locomotor responses to subcutaneously-injected MK801 24 h later.

The ketamine pretreatment significantly changed the total distance travelled in cm by rat pups during the 2 h test session, in comparison with rat pups not treated 24 h

previously with ketamine, but the direction of change differed between 21-day-old and 31-day-old rat pups (Fig. 3-11). In 21-day-old rat pups pretreated with ketamine/xylazine, the MK801 doses of 0.1 and 0.5 mg/kg induced debilitating ataxia in more animals than in the pretreatment group, thereby decreasing the total distance travelled relative to the non-pretreated control group. Statistical comparisons with pretreatment condition and dose as factors in a two-way ANOVA confirmed significant effects of pretreatment condition [$F(1,95)<9.59$, $P<0.01$] and dose [$F(4,95)=18.17$, $P<0.001$] as well as a significant condition x dose interaction [$F(4,95)=4.62$, $P<0.01$]. In 31-day-old rat pups pretreated with ketamine/xylazine, the responses to MK801 differed with respect to gender [$F(1,82)=10.65$, $P<0.01$], dose [$F(4,82)=17.42$, $P<0.001$] as well as a significant gender x condition x dose interaction [$F(4,82)=2.74$, $P<0.05$]. In males, the total distance travelled in response to both 0.5 and 1.0 mg/kg MK801 was higher in pretreated rats than in non-pretreated rats. In females, the total distance travelled was higher in pretreated rats than in non-pretreated rats only following 0.5 mg/kg MK801. Thus, pretreatment with ketamine/xylazine essentially augmented the motor effect of for MK801 administered 24 h later in both 21- and 31-day-old rat pups such that 21-day-olds exhibited more ataxia, while 31-day-olds exhibited more locomotor activation than in conditions without pretreatment.

Discussion

MK801 injected into the nucleus accumbens of developing and adult rats increased locomotion in a dose- and age-dependent manner. Given that the pharmacological action of MK801 is to block NMDA receptor-associated ion channels that have already been opened by endogenous glutamate, the ontological changes in

MK801-induced activity are likely to reflect maturational changes in glutamate activity in the limbic-motor circuitry mediating the locomotor effects of MK801.

MK801 in the nucleus accumbens blocks the activity of glutamate projections from the limbic neocortex, hippocampus and amygdala in order to influence locomotion. Glutamate interacts with dopamine in the nucleus accumbens in order to alter the activity of efferent GABA projections which target motor output regions. It has been proposed that there are two opposing efferent pathways from the striatum (Alexander and Crutcher 1990, Gerfen 1992, Smith and Bolam 1990). Glutamate stimulation of the excitatory output pathway would increase motor activity, glutamate stimulation of the inhibitory output pathway would decrease motor activity. Dopamine would excite the excitatory pathway and inhibit the inhibitory pathway, increasing locomotion either way. This idea has been extended to include parallel dual output pathways from the ventral striatum (including the nucleus accumbens) to the ventral pallidum, and could explain how activity in the ventral striatum and the pharmacological manipulation thereof could influence locomotion particularly in response to novelty (Carlsson 1993, Svensson et al. 1992a and b, 1994). In familiar situations, the inhibitory pathway would be tonically activated, receiving steady glutamate inputs from the cortex and producing a resting state of low motor activation. Antithetically, during times of intense sensory stimulation, the excitatory pathway would be phasically activated by both glutamate and dopamine release, increasing motor activity. The switching mechanism from one pathway to the other has yet to be defined but may be related to the source of glutamate input to the accumbens or the balance between glutamate and dopamine inputs.

This dual output hypothesis can be used to interpret the locomotor activity of rats in the present experiment. First, following the minor stress of dH_2O injection and placement in a novel environment, phasic activation of the excitatory route would be triggered via the stress- and novelty-induced increases in dopamine and glutamate release in the nucleus accumbens (Abercrombie et al. 1987, Bradberry et al. 1991, Hooks et al. 1992, Horger and Roth 1995, Moghaddam 1993, Rebec et al. 1997). As the animals explored and subsequently habituated to the test monitor, activity would be inhibited by a return to phasic stimulation of the inhibitory route. Following MK801 injection, the phasic activation of the excitatory pathways would increase locomotion before the drug was active, but MK801 would then block the tonic activity of glutamate at the inhibitory pathway. Thus, MK801 would not affect the initial exploratory activity but would block the later habituation to the test monitor, thereby increasing locomotion until the drug was metabolized. This hypothesized neural mechanism might not be mature in 11-day-old rat pups, only 37% of which exhibited any response at all to the MK801. In 21-day-old pups, the circuitry appeared to be supersensitive to MK801, as shown by the high magnitude of activation they exhibited, but 31-day-old and adult rats appeared less sensitive to MK801 because they had lower levels of activation in response to intra-accumbens MK801 injections. Twenty and 40 μg MK801 increased locomotion in 31-day-old and adult rats, but these doses, administered in higher volumes than lower doses, may have spread into the lateral ventricles.

Eleven-day-old rat pups showed little response to MK801. Only 37% exhibited any notable motor response to either 3 or 10 μg MK801, compared with 80 to 100% of

older pups exhibiting robust locomotion. The motor activity of the 11-day-olds consisted of sporadic obstinate progression-like activity following injection of either 3 or 10 μg MK801. For periods of 5-15 min, these pups would locomote in the perimeter of the activity monitor until reaching a corner, where they would maintain their heads in the corner, continue stepping with fore- and hindlimbs, often progressing up the wall, and sometimes ending the bout by rolling onto their dorsal surfaces, apparently unable to right themselves. Their activity differs from that of 11-day-old pups injected subcutaneously with MK801. Those pups locomoted in short bouts but did not exhibit obstinate progression or wall-climbing (see Chapter 2). The obstinate progression is very similar, though, to the behavior of reserpinized adult rats given a combination of MK801 and the α_2 noradrenergic antagonist, clonidine (Carlsson and Svensson 1990) and to the activity of decorticate cats (Villablanca 1976). Wall-climbing is a classic response to dopamine agonists or stress in rats approximately 10 days of age (Barrett et al. 1982, Johanson and Hall 1982, Reinstein et al. 1978, Shalaby and Spear 1980, Stehouwer and Campbell 1980). These similarities and contrasts may provide information as to which neural mechanisms of MK801-induced locomotion are immature in the present 11-day-old pups.

Any aspect of the limbic-motor circuitry thought to mediate MK801-induced locomotion in adult rats could be immature in the 11-day-old pup. The levels of endogenous glutamate in the nucleus accumbens may not be sufficient to open the NMDA-associated ionophore. The prefrontal cortex and hippocampus mature relatively late in ontogeny (Angevine, Jr. 1975, Alexander and Goldman 1978, Benes 1989,

O'Keefe and Nadel 1978), making it conceivable that their glutamatergic projections into the nucleus accumbens are not yet mature in the 11-day-old rat. Anatomical analysis of the development of these specific inputs has not been carried out, but Campochiaro and Coyle (1978) reported an increase in glutamate levels and high-affinity glutamate uptake in the striatum of rat pups at approximately 14 days of age. They interpreted these findings as reflecting the ontological onset of glutamate release into that region. Furthermore, cognitive tasks thought to involve the medial prefrontal cortex and hippocampus are not performed accurately by young rat pups (Green and Stanton 1989), suggesting that these brain regions are not yet mature. The resemblance between decorticate cats and 11-day-old rats given MK801 also supports the contention that a lack of cortical inputs to the accumbens could be involved in obstinate progression. Furthermore, lesions of the hippocampus in neonatal rat pups have no effect on motor activity until after puberty, implying that the hippocampus does not become adult-like in function with respect to locomotion or even responses to stress until late in ontogeny (Flores et al. 1996, Wan et al. 1996). However, those findings show influence of the hippocampus on locomotion after postnatal day 35 and before day 56, whereas our findings show adult-like locomotor responding to intra-accumbens MK801 already at 31 days of age. The age-related discrepancy may be attributable to differences between effects of chronic lesions and acute receptor blockade.

On the other hand, mechanisms postsynaptic to glutamate inputs in the nucleus accumbens may not yet be mature in the 11-day-old rat. NMDA receptors may not exist in sufficient density, may not be associated with the appropriate intracellular mechanisms, or may not be present on the appropriate output neurons. The lack of

consistent motor responding to MK801 could represent a functional "pre-coupling" of glutamate and nucleus accumbens efferents, in which case the sporadic motor response to MK801 exhibited by 11-day-olds could be mediated by blockade of the NMDA ionophore in other basal ganglia regions or even in the spinal cord. A "pre-coupling" of glutamate with nucleus accumbens outputs might reveal greater influence of other neurotransmitters on behavior in ontogeny, which might explain why MK801-induced behavior in 11-day-old rat pups resembles that of adult rats also depleted of dopamine and given a noradrenergic agonist.

While it could be postulated that nuclei to which the efferent fibers of the nucleus accumbens project are not developed enough to facilitate locomotion, intra-accumbens injections of quinpirole can increase locomotion substantially in 11-day-old pups (Frantz and Van Hartesveldt 1995). Therefore, output from the nucleus accumbens is at least capable of producing locomotion at 11 days of age.

Alternatively, glutamate in the nucleus accumbens of 11-day-olds may selectively activate the excitatory output pathway. In this case, blockade of glutamate receptors in 11-day-olds would only decrease locomotion. Several ideas refute this concept. The novel environment of the current test conditions elicited very low activity in the control group, suggesting that endogenous glutamate does not normally activate the excitatory pathway to any great extent. As a result, locomotor suppression could not be detected, even if it had occurred due to blockade of the excitatory pathway by MK801. Testing rats in their more active dark phase or in the presence of milk might raise the baseline activity enough to provide a contrast with glutamate receptor blockade. In the presence of milk, MK801 did decrease mouthing in 3-4-day-old rat pups, but in doses that may

have produced interfering behaviors such as locomotion or ataxia (Rajachandran et al. 1991). Regardless, the bouts of MK801-induced activity recorded in the present study certainly were not examples of locomotor suppression. Pilot experiments in this laboratory also showed that NMDA injected into the nucleus accumbens of 11-day-old pups did not increase locomotion either, making it doubtful that NMDA receptor activity selectively activates the excitatory pathway early in ontogeny.

Finally, it is possible that the behavioral arousal exhibited by the 11-day-olds injected with MK801 is the typical motor response for pups of this age and that it reflects the same degree of arousal as does more consistent locomotion in older pups. In the home cage, 11-day-old pups still exhibit bouts of two main behaviors: suckling and huddling. When activated in the absence of a dam, suckling is not an option, so the pups may exhibit behaviors akin to huddling, including short, sporadic bouts of motor behavior, consisting of forepaw treading and almost "nuzzling" the corner of the test box. Injections of other motor activating drugs, such as amphetamine (Barrett et al. 1982) or the D_2/D_3 dopamine receptor agonist, quinpirole (Frantz and Van Hartesveldt 1995, Van Hartesveldt et al. 1994), also show that the activity of 11-day-olds is sporadic and occurs at different times across a test session for individual pups and can include wall-climbing in corners. Thus, there are many possible explanations for the lack of consistent motor responding in 11-day-old rats given intra-accumbens MK801 injections. The possibilities are not mutually exclusive.

Whereas 11-day-old rat pups exhibit low, inconsistent levels of motor response to MK801 and other stimulant drugs, 21-day-old rat pups typically exhibit high activation. Generally, 21-day-old pups exhibit high locomotor responses to novelty (Campbell et al.

1969) and in the present study, they exhibited the highest magnitude of MK801-induced locomotion, with the shortest latency to onset and the longest duration, compared with rats of other ages. Such characteristics imply that the neural mechanisms mediating novelty- and MK801-induced activation are intact and even supersensitive at this age. The locomotor circuitry may be especially prone to activation in 20-day-old rats in order to promote exploratory activity during this developmental stage in which rat pups would be close to weaning and independence from their dam and littermates.

Indeed, at least in the striatum, several aspects of glutamate function reach adult levels during the second and third postnatal weeks and could be supersensitive early in their development. As noted previously, the level of endogenous glutamate in the striatum doubles during the second postnatal week, as does high affinity glutamate uptake; perhaps because afferent fibers start releasing glutamate into the accumbens at that time (Campochiaro and Coyle 1978). Beginning at approximately the same time, a plateau of [^{3}H] MK801 binding just above adult levels is seen in both dorsal and ventral subregions of the striatum (Subramaniam and McGonigle 1994). A slight overshoot of [^{3}H] glutamate binding at NMDA receptors is noted at the same stage, but otherwise, NMDA receptor levels in the striatum are steady from postnatal day one to adulthood (Insel et al. 1990). In other limbic brain regions, though, such as the cortex and hippocampus (Insel et al. 1990), as well as motor regions (Greenamyre et al. 1987), a transient overexpression of NMDA receptors is robust at approximately 20 days of age. Together, these biochemical assays confirm that maturation of the striatal glutamate system is especially extensive during the second and third weeks in ontogeny and similar development of glutamate inputs to the nucleus accumbens could conceivably mediate

the sensitivity of 21-day-old rats to the locomotor activating effects of intra-accumbens MK801.

Not only were 21-day-old pups highly sensitive to the locomotor-activating effects of MK801 but also they were severely debilitated in response to the 20 μg dose of intra-accumbens MK801. The ataxia was typical of that induced by peripherally-injected MK801 (see Chapter 2). It could have resulted from overstimulation of motor nuclei due to blockade of all the ion channels associated with the inhibitory output pathway from the nucleus accumbens. Alternatively, it might have been produced by a high-dose effect of MK801 on release of another neurotransmitter, such as acetylcholine (Al-Khatib et al. 1995). Otherwise, it could be hypothesized that the ataxia was mediated by drug diffusion to regions around the nucleus accumbens. However, specific placement of a lower dose of MK801 in the mid-striatum of 21-day-olds, the brain region to which MK801 was most likely to diffuse, produced locomotor activation, not ataxia. Furthermore, a 20 μg dose of MK801 in adult rats failed to produce ataxia when injected into the striatum (Al-Khatib et al. 1995).

If high levels of glutamate release, a high density of NMDA receptors or overabundant postsynaptic connections mediate the supersensitivity of 21-day-old rat pups to intra-accumbens MK801, then stabilized levels of glutamate release, programmed cell death, synaptic pruning or receptor elimination could contribute to the decreased activational effect of MK801 in 31-day-old and adult rats. Thirty-one-day-old pups were activated by the 10 μg dose of MK801 but not as much as 21-day-olds, and adult rats were not even significantly affected by this dose. The absence of locomotor responding

to 10 μg MK801 placed in the nucleus accumbens of adult rats confirms the findings of Al-Khatib et al. (1995). Again, these age-related changes in responding are fairly typical of those induced by other locomotor-activating drugs (Frantz et al. 1996, Spear and Brake 1980, Van Hartesveldt et al. 1994). Generally, 31-day-old pups nearing sexual maturity may take on a more "cautious" exploratory behavior, being less active than 21-day-olds. By adulthood, a novel environment may not be "threatening," may elicit less exploratory attention, and may garner lower levels of locomotor activation. These results are slightly different from the peripheral effects of MK801 (see Chapter 2). Thirty-one-day-olds were less active than adults in that case, probably reflecting differences in activity of MK801 outside the nucleus accumbens. Also, the striking gender differences seen in response to peripheral injections of MK801 were no longer recorded with intra-accumbens injections, showing that gender differences in response to MK801 are not mediated in the nucleus accumbens.

A less likely explanation than those described above for the ontological differences in response to MK801 is that slight differences in anatomical placement of the drug determined the locomotor responses. The injections were given most laterally to 11-day-olds and progressively more medially from 21-day-olds to 31-day-olds to adults. Within the nucleus accumbens, subregions termed the core, shell and rostral pole have been delineated based on the binding of various proteins (Zahm and Brog 1992, Zahm and Heimer 1993). The afferent and efferent connections of the subregions indicate that the core is generally motoric in function; the shell more limbic and the rostral pole, a cross between the two. These subregions are increasingly recognized as separate

functional units (Deutch et al. 1993). The core, shell and rostral pole delineations were not defined in the present study for several reasons. First, drug diffusion was assumed to enable the drug to block NMDA channels in core, shell and rostral pole. Second, the ontogeny of these anatomical differentiations has not been investigated, so it is not clear when they are first present or what their configuration is in the developing rat. Third, most drug placements in the present study would have been defined as rostral pole placements and this placement would enable MK801 to affect both limbic and motor circuitry. However, it is possible that none of these factors was influential and that the slight variations in injection placement across ontogeny actually determined the locomotor response to MK801. Maldonado-Irizarry and Kelley (1994) injected the competitive glutamate antagonist, AP5, into regions corresponding to either the core or the shell and showed that novelty-induced activity in adult rats was decreased by injections into the core region but was increased by placements in the shell. Results from 11-day-old pups in the present study corroborate this finding in that the pups defined as responders to MK801 had the most lateral placements in comparison with other 11-day-olds. Such placement may have blocked NMDA channels in the lateral shell region, leading to increased activity. Yet even their activity was sporadic and low in magnitude. On the other hand, 21-day-old rat pups in the present study confound the core vs. shell distinction of Maldonado-Irizarry and Kelley because these pups showed the highest levels of activation but had the most central, core-like placements. Similarly, 31-day-old pups and adults fail to corroborate the differentiation because they exhibited successively

less activity in response to MK801 and their placements were, if differentiable, more medial and therefore more shell-like.

Rostral and caudal regions of the nucleus accumbens have also been delineated with respect to locomotion induced by dopaminergic drugs. Essman et al. 1993 showed that a combination of D_1 and D_2/D_3 dopamine receptor agonists induced more activity from the caudal-central region, as compared with more rostral or caudal drug placements. On the contrary, observations from a few subjects eliminated from the present study due to placements outside the acceptable range, showed that high levels of activity could be induced even by the most rostral placements of MK801 in the accumbens, but more caudal placements failed to produce locomotion.

The 20 and 40 μg doses of MK801 substantially increased the activity of 31-day-old and adult rats, but these doses of MK801 were given in greater volumes due to the low solubility of MK801. Thus, they probably diffused into the lateral ventricles, exerting their stimulatory effects from other brain regions. When 20 μg MK801 was injected directly into the lateral ventricles of adult rats, it increased locomotion to the same degree as intra-accumbens MK801, but with a much shorter latency to activation. This finding may indicate that following injections aimed at the accumbens, the drug took longer to diffuse into the ventricles from which it was distributed to produce high levels of locomotor activation from other brain regions. This is not to say that MK801 injected into the nucleus accumbens itself cannot alter locomotor activity. Al-Khatib et al. (1995) recorded locomotor activation in adult rats receiving intra-accumbens injections of 20 μg MK801 that were administered from an off-vertical angle that prohibited diffusion into

the ventricles. Also, in the present study, when 10 μg MK801 was injected into 21- and 31-day-old pups in a lower volume, histological analysis did not indicate that extensive drug diffusion had occurred, and these rats exhibited locomotor activation. The ICV injections merely suggest that an ICV placement of MK801 produces higher levels of activation, more rapidly, than intra-accumbens placements. From the lateral ventricles, MK801 is likely to have been further distributed to the hippocampus, amygdala, posterior piriform cortex and other circumventricular organs, based on the distribution of intra-ventricularly-injected ibotenic acid described by Bardgett et al. (1997). Several of those regions are rich in NMDA receptors.

An additional methodological concern with the higher doses of MK801 is that the histology following their injection in 21-, 31-day-old and adult rats showed that the acidity of the solution altered the brain tissue so as to decrease the ability of the thionin stain to dye cells in a column around and dorsal to the injection site. It might be postulated that this tissue alteration formed an acute lesion that led to the motor activation shown in response to the higher doses of MK801, but the tissue alteration and motor activation were dissociated; rats injected with the acidic solution not containing MK801 did not exhibit locomotor activation or ataxia but their tissue did show the same absence of staining around the injection site. Furthermore, if the effect of the acidic solution had been effectually a lesion of the nucleus accumbens tissue, then rats would be expected to exhibit hyperlocomotion and normal levels of rearing in a novel environment, as do rats with ibotenic acid lesions of the nucleus accumbens, albeit more than 10 days after

induction of the lesion (Burns et al. 1996, Kafetzopoulos 1986). Such behavior was not observed.

One final procedural concern deserves discussion. The rats 21 days of age and older were anesthetized with a ketamine/xylazine solution for the cannula-implantation surgery approximately 24 h prior to testing of MK801-induced locomotion. Ketamine acts at the same binding site in the NMDA receptor-associated ion channel as MK801 and therefore might have influenced the response to MK801. Other anesthetics have proven far less safe and effective for rat pups in this laboratory, so instead of using a different anesthesia, the possibility that ketamine could affect motor responding to MK801 was investigated by injecting the anesthetics in 21- and 31-day-old rats and then testing their responses to peripherally-injected MK801 24 h later. The motor suppressant effects of the anesthesia did not persevere through the locomotor testing the following day, as shown by the saline-injected control groups' comparable levels of activity, regardless of whether they had anesthesia-pretreatment or not. In 21-day-old pups, the anesthesia pretreatment increased the proportion of rats exhibiting severe ataxia, thereby decreasing the total distance traveled by the pretreated group overall. In 31-day-old pups, the locomotor-activating effects of MK801 were potentiated by anesthetic pretreatment. The effect occurred across a wider dose-range in males than females, in contrast to the usual higher sensitivity of female rats to sensitization and to the locomotor activating effects of MK801. If the anesthesia had the same effect on activity induced by intra-accumbens MK801 as it did on peripheral MK801 injections, then the activity of 21-day-olds would have been even greater in magnitude and the activity of 31-day-olds and adults would have been even lower.

A locomotor-potentiating effect of anesthetic pretreatment is generally corroborated by experiments with MK801 and PCP in which treatment with 0.4 mg/kg MK801 or 3.2 mg/kg PCP potentiated the locomotor response to the same injection the following day (Wedzony et al. 1993, Xu and Domino 1994). However, a lower dose of 0.18 mg/kg MK801 did not sensitize adult females to locomotor activation the following day (Xu and Domino 1994). Whether or not that pretreatment caused ataxia the following day was not reported. The effects of ketamine pretreatment also contradict a report by Gorter et al. (1992) in which 0.25 mg/kg MK801 administered daily from postnatal days 8 to 19 still increased locomotion on day 19. The sensitizing effects of ketamine could, therefore, be different from those of MK801.

It is important to note that the conditions of peripheral injection of MK801 after anesthetic pretreatment may not be comparable to the intra-accumbens MK801 injections. Changes in response to peripheral injections of MK801 could reflect changes in the liver metabolism of MK801, changes that might even generalize to other anesthetics. The finding that 21-day-old pups travelled farther and were more ataxic than other age-groups, even after peripheral injections without anesthetic pretreatment, demonstrates that it is not simply the anesthesia causing 21-day-olds to be more sensitive to the activating and debilitating effects of intra-accumbens MK801.

There are several conclusions to be made from these experiments. First, the lack of consistent responding in 11-day-old pups, in which corticofugal fibers may not yet be mature, and the high magnitude of responding in 21-day-old pups, in which glutamate transmission is florid, are consistent with the hypothesis that glutamate inputs to the nucleus accumbens mediate ontological changes in responses to intra-accumbens MK801.

Second, the lack of gender differences in response to intra-accumbens MK801 supports a metabolic or at least extra-accumbens mechanism for gender differences in response to peripherally-injected MK801. Finally, methodological advancements are necessary to define more precisely the role of glutamate in the nucleus accumbens of 30-day-old or adult rats.

The clinical significance of this research relates mainly to schizophrenia, a human disorder of cognitive, emotional and motor behaviors. The condition involves abnormal development of mesocorticolimbic feedback loops between the ventral tegmental area, nucleus accumbens, cortex and hippocampus (Doran et al. 1988, Walker 1994, Weinberger 1988, Weinberger et al. 1988). The neurotransmitters glutamate and dopamine are major determinants of activity in these pathways and, as such, they are targets for the pharmacological treatment of this disorders (Halberstadt 1995, Moghaddam 1994, Olney and Farber 1995a and b, Wachtel and Turski 1990).

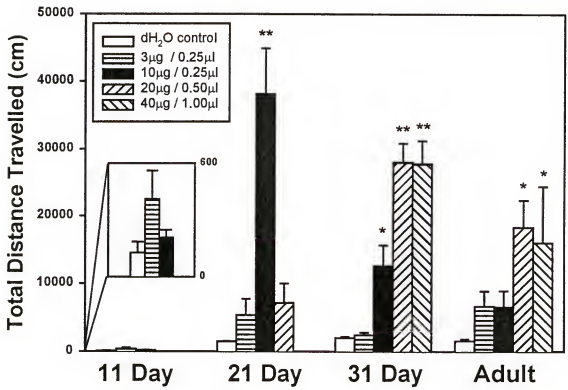

Figure 3-1. Locomotion elicited by intracerebral MK801.

Total distance (cm) travelled in a 2 h test session by rats of several different ages, following intracerebral injection with various doses of MK801. Error bars represent the standard error of the mean. Significant differences from dH_2O-injected controls are indicated (**P<0.01, *P<0.05).

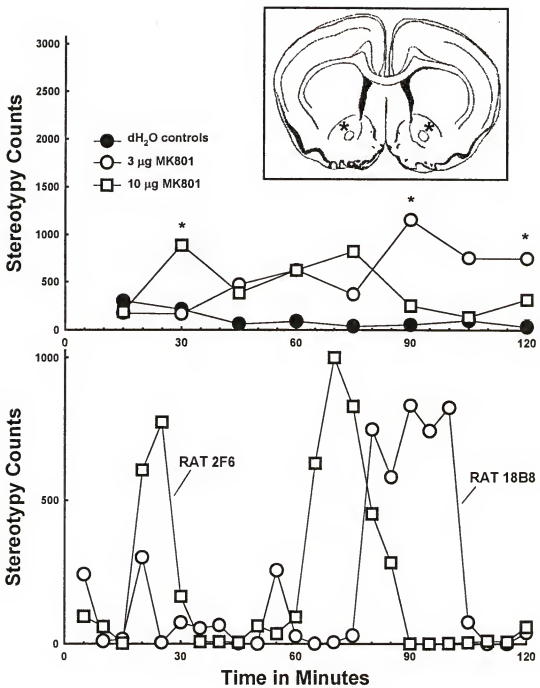

Figure 3-2. Stereotypy counts for eleven-day-old rat pups.

A) Stereotypy counts for the 11-day-old rat pups that exhibited a motor response to intra-accumbens injection of 3 or 10µg MK801. Significant differences from dH_2O-injected control groups are shown (*$P<0.05$); B) Stereotypy counts for two individual rat pups injected with 3 or 10µg MK801 into the nucleus accumbens; Inset) Approximate location of MK801 injection into the nucleus accumbens.

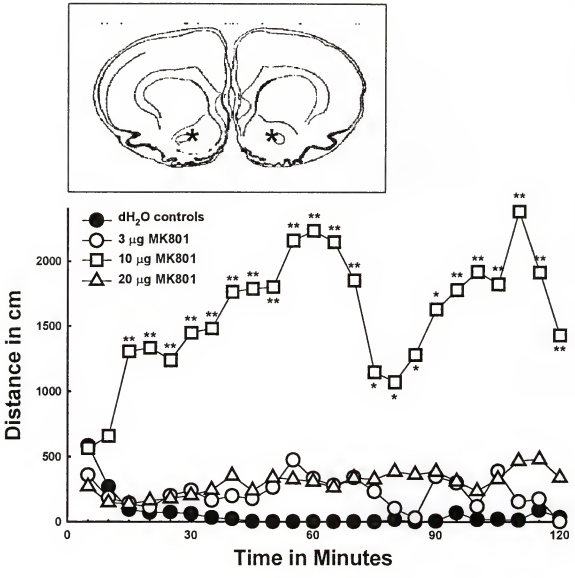

Figure 3-3. Distance travelled by twenty-one-day-old rat pups.

Distance travelled (cm) by 21-day-old rat pups following intra-accumbens injection of various doses of MK801. Significant differences from the dH_2O-injected control group are shown (**P<0.01, *P<0.05). Error bars have been omitted for clarity. Inset shows approximate location of MK801 injection into the nucleus accumbens.

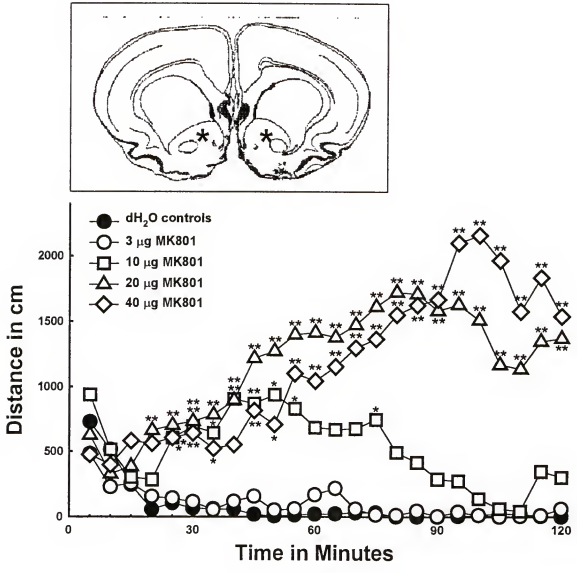

Figure 3-4. Distance travelled by thirty-one-day-old rat pups.

Distance travelled (cm) by 31-day-old rat pups following intracerebral injection of various doses of MK801. Significant differences from the dH_2O-injected control group are shown (**P<0.01, *P<0.05). Inset shows approximate location of MK801 injections into the nucleus accumbens.

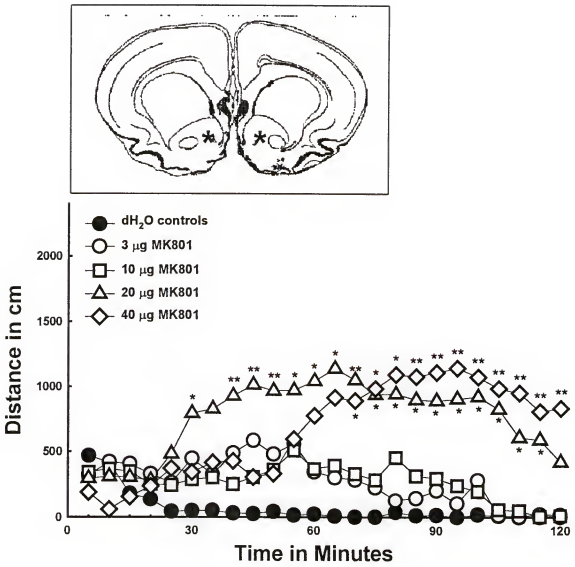

Figure 3-5. Distance travelled by adult rats.

Distance travelled (cm) by adult rats following intracerebral injection of various doses of MK801. Significant differences from the dH_2O-injected control group are shown (**P<0.01, *P<0.05). Inset shows approximate location of MK801 injections into the nucleus accumbens.

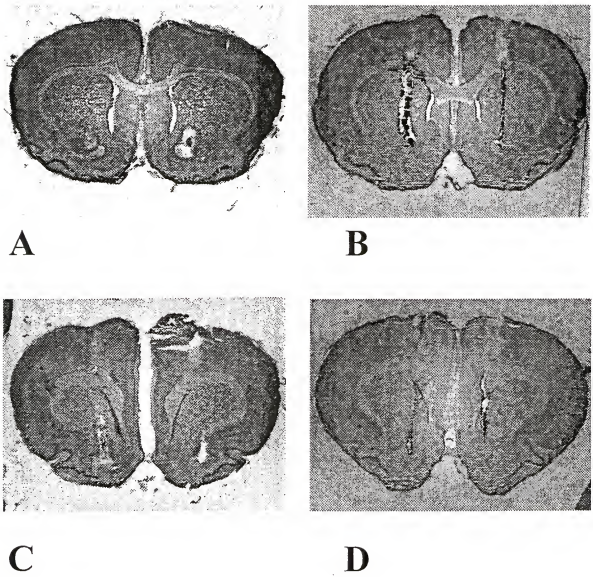

Figure 3-6. Photomicrographs of intra-accumbens injection placement.
A) Brain slice from 11-day-old rat showing location of intra-accumbens drug injection; B) brain slice from 21-day-old rat; C) brain slice from 31-day-old rat; D) brain slice from adult rat.

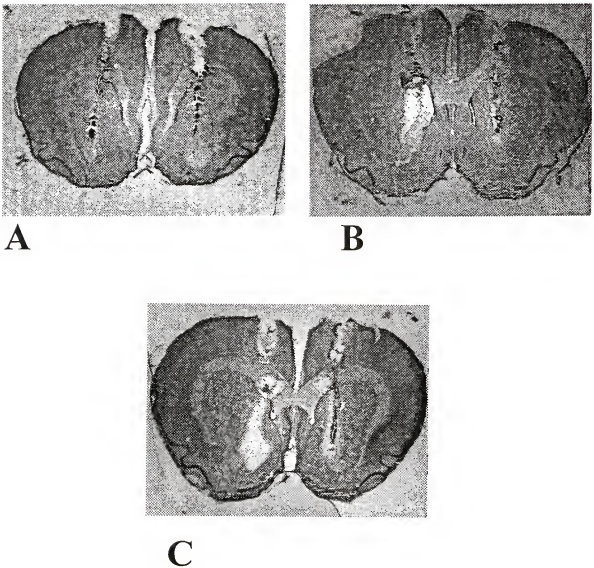

Figure 3-7. Photomicrographs showing injection of 20 μg MK801 in 0.5 μl solution.
A) Brain slice from 21-day-old rat showing intra-accumbens injection of 20 μg MK801 in 0.5 μl; B) brain slice from 31-day-old rat showing acid-induced lack of staining and possible diffusion of 20 μg MK801 into the lateral ventricles; C) brain slice from adult rat under the same conditions.

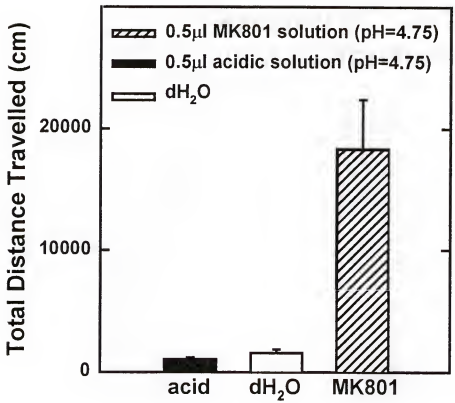

Figure 3-8. Comparison between acid-, dH_2O-, and MK801-induced locomotion.
Total distance (cm) travelled by adult rats in a 2 h test session following intracerebral injection of 0.5µl of either and acidic solution (pH=4.85), dH_2O, or 0.12M MK801 solution (20µg dose, pH=4.85).

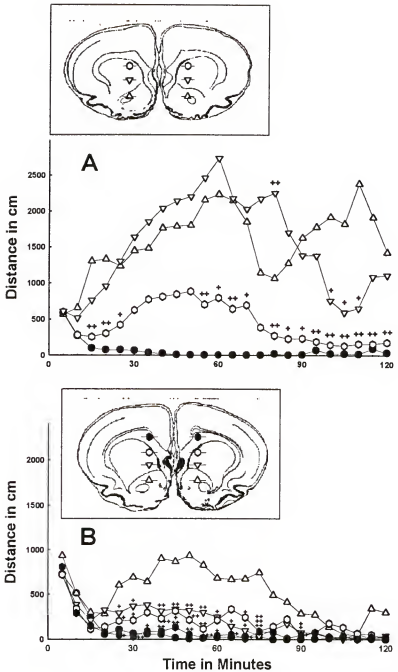

Figure 3-9. Specificity of injection placement in twenty-one and thirty-one-day-old rats. A) Distance travelled (cm) by 21-day-old rat pups following injection of 10μg MK801 to the nucleus accumbens, mid-striatum or dorsal striatum; B) The same measure for 31-day-old pups, including a fourth intra-cerebral injection placement near the corpus callosum. Insets) Approximate location of MK801 injections to the nucleus accumbens and striatum. Significant differences between the intra-accumbens injection site and the other sites are shown (++P<0.01, +P<0.05). Closed circles represent activity of control groups injected with dH_2O into the nucleus accumbens.

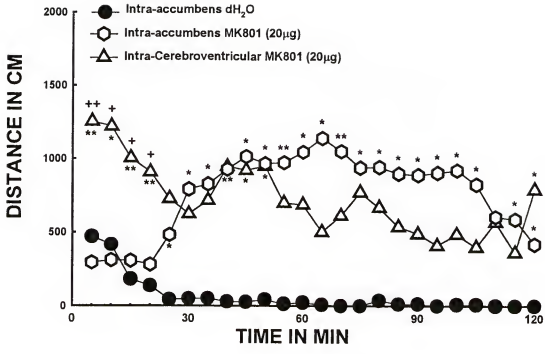

Figure 3-10. Comparison between intra-ventricular and intra-accumbens MK801.

Distance travelled (cm) by adult rats following injection of 20μg MK801 or dH_2O into either the nucleus accumbens or the lateral ventricles. Significant differences between the intra-accumbens and ICV injections of MK801 are shown (++P<0.01, +P<0.05), as are differences between the MK801 injections (either site) and the dH_2O injections are shown (**P<0.01, *P<0.05).

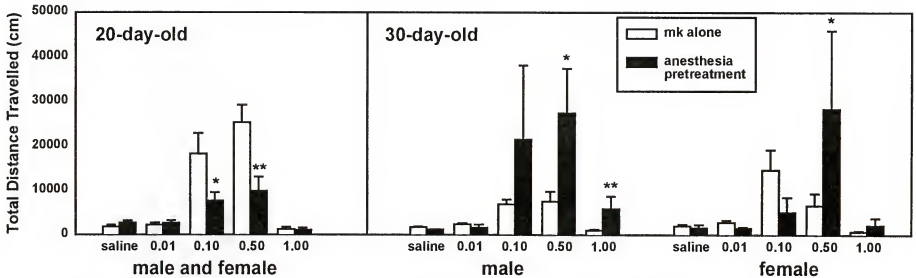

Figure 3-11. Effects of anesthesia pretreatment on locomotion induced by subcutaneously-injected MK801.

A) Total distance travelled (cm) by 21-day-old rats in a 2 h test session after injection of MK801 in various doses either pretreated with ketamine/xylazine anesthesia (filled bars) or not pretreated (open bars); B) The same measure for 31-day-old rats. Significant differences between the pretreatment conditions are shown (**P<0.01, *P<0.05).

CHAPTER 4

GENERAL DISCUSSION

The most striking result in these experiments was the robust potentiation of locomotor activity elicited by exposure to a novel environment 60 min after subcutaneous injection of the NMDA receptor antagonist, MK801. The level of locomotion elicited by the interaction between these two stimuli was higher than the sum of activation produced by either stimulus alone. Interactions between MK801 and novelty-induced release of stress hormones may have produced synergistic effects on locomotion. This effect occurred in rats 20 days of age and older, but not in 10-day-old rats. Therefore, some aspect of glutamate transmission or stress hormone release in response to novelty appears to be immature in 10-day-old rat pups. Subsequent to the potentiated locomotion, an habituation-like decline in locomotion was observed, except in adult female rats, none of which habituated substantially to the test monitor.

The similarity of responses to peripheral and intra-accumbens MK801 injections, in terms of both the quality of motor behavior and the ontological changes in magnitude of drug-induced locomotion, indicate that the peripheral effects could be mediated at least in part by the activity of MK801 in the nucleus accumbens, even in developing rats. The similarity also indicates that the differences in sensitivity to MK801 in response to peripheral drug injections are probably not mediated by ontological differences in biotransformation of the drug, but rather reflect changes in nucleus accumbens circuitry.

The gender-dependent effects of MK801 were also notable. In the no-delay condition, both 30-day-old and adult female rats exhibited higher levels of activation in response to 0.1 mg/kg MK801 than males. Also in the no-delay condition, adult female rats exhibited ataxia with akinesia for a longer period of time in response to the 0.5 mg/kg dose of MK801. In the delay condition, adult female rats showed the same level of potentiated locomotion as males, but females did not exhibit the subsequent habituation-like decline in activity. The high sensitivity of females to MK801 and environmental novelty were most likely mediated by both their less efficient metabolism of MK801 and higher levels of stress hormone release in response to environmental novelty. The absence of gender differences in response to intra-accumbens MK801 injection shows that the higher sensitivity of females in response to peripheral MK801 injection was not mediated in the nucleus accumbens but rather occurred elsewhere in the central nervous system or via peripheral mechanisms.

Interactions Between Glutamate and Dopamine

Whereas MK801 interacts with the novel environment to increase locomotion, the D_2/D_3 dopamine receptor agonist, quinpirole, interacts with the novel environment to decrease locomotion. Whereas MK801 should block the function of endogenous glutamate, quinpirole should mimic the function of endogenous dopamine. If interactions between novelty and these drugs are mediated in the nucleus accumbens, then the conclusion should be that glutamate and dopamine both decrease locomotor activity when released in the nucleus accumbens. Years of research showing that dopamine in the nucleus accumbens can facilitate locomotion, demonstrate this conclusion to be too simplistic. More recent findings that NMDA itself in the nucleus accumbens can increase

locomotion, demonstrate again that the idea is too simplistic. Furthermore, such a conclusion does not account for the receptor subtype specificity of quinpirole and MK801, which makes their pharmacological activity different from that of the endogenous transmitter. Yet based on these ideas, many hypothesized mechanisms for the synaptic interactions between glutamate and dopamine in the nucleus accumbens have been proposed. At present, pieces of evidence support each hypothesis but no crucial findings prove any theory. To follow is a description of several of these concepts and a discussion of research from this laboratory that addresses several aspects of the hypotheses.

Three major possibilities exist for the interactive activity of glutamate and dopamine in the nucleus accumbens: 1) postsynaptic activity convergent on medium spiny output neurons; 2) presynaptic activity at heteroreceptors on terminals releasing the other neurotransmitter; and 3) presynaptic activity at autoreceptors. In all likelihood, the neurotransmitters act at all the possible sites at different times depending on as yet unidentified factors that "switch" activity from one location to another.

The research of Mogenson, Wu and colleagues (e.g. Wu et al. 1993b) supports all three possibilities, and defines the level of glutamate release as the "switch" factor that determines the sites of action of dopamine and glutamate in a given situation. These investigators suggest that when excitatory impulses from glutamatergic inputs are high, as in the case of exposure to a novel environment, dopamine agonists (at the D_2 dopamine receptor subtype in particular) act presynaptically. The dopamine agonist could act on glutamate terminals to decrease glutamate release, as well as on dopamine autoreceptors to decrease dopamine release, thereby blocking novelty-induced locomotion. When the

environment is familiar, dopamine agonists are said to act at postsynaptic receptors to increase locomotion. Wu and colleagues predict that some aspect of specifically the hippocampal glutamatergic input provides the switch from pre- to postsynaptic activity of the dopamine agonist. For glutamate activity, they suggest that glutamate agonists act presynaptically at dopamine terminals to increase locomotion, an idea supported by their finding that a 6-hydroxy-dopamine lesion of dopamine terminals decreases locomotion induced by glutamate agonist-induced locomotion.

On the other hand, the work of Ougazzal et al. (1994) and Svensson et al. (e.g. 1994) support the postsynaptic integration of glutamatergic and dopaminergic effects. They have shown that decreasing dopamine release in the nucleus accumbens via 6-hydroxy-dopamine lesion or reserpinization does not completely abolish MK801-induced locomotion, indicating that endogenous glutamate must act at post-synaptic receptors rather than presynaptic dopamine terminals. Conversely, injection of a dopamine antagonist does complete abolish MK801-induced locomotion, presumably by blocking postsynaptic receptors, indicating that dopamine receptor activation is a necessary component of glutamate-mediated activity. In addition, Svensson et al. (1992a) have shown that subthreshold doses of an NMDA receptor antagonist and a dopamine agonist together increase locomotion, even in dopamine-depleted mice. They conclude that MK801 has activity independent of, but potentially synergistic with, dopamine release. Glutamate itself presumably has the same potential functions but would exert the opposite effects. Svensson and colleagues have further proposed the dual-output hypothesis for the postsynaptic activity of both dopamine and glutamate in the nucleus accumbens (e.g. Svensson et al. 1994, Carlsson 1993).

Anatomical data do not resolve the issue. The appearance of excitatory glutamatergic synapses on dopaminergic terminals has not been demonstrated, suggesting that presynaptic interactions might not occur. Rather, an observed convergence of glutamate and dopamine inputs on the same medium spiny output neurons supports a postsynaptic interaction between glutamate and dopamine. Close apposition of the glutamatergic and dopaminergic terminals on the medium spiny output neurons has been recorded, though, and could provide a means for atypical presynaptic interaction between glutamate and dopamine (Sesack and Pickel 1990, 1992).

The hypothesis that presynaptic activity of dopamine agonists at autoreceptors mediates the novelty-dependent dopamine agonist-induced locomotor suppression is not supported by research from this laboratory (Frantz and Van Hartesveldt 1994, 1995, Van Hartesveldt et al. 1992). For example, intra-accumbens injections of quinpirole suppress novelty-induced locomotion only in doses too high to remain autoreceptor selective. Furthermore, dopamine autoreceptors are present and biochemically functional in the nucleus accumbens of 5-day-old rat pups (Andersen and Gazzara 1994), but dopamine agonist-induced locomotor suppression in response to intra-accumbens injection of quinpirole does not occur until the second or third postnatal week.

The hypothesis that glutamate acts presynaptically at dopamine terminals is not supported by the results of the present studies or those of many other experiments. If glutamate acts presynaptically at dopamine terminals to increase locomotion, then a glutamate antagonist would presumably act presynaptically to decrease locomotion. Therefore, all the studies in which an intra-accumbens injection of a glutamate antagonist increases locomotion refute this hypothesis.

Experimental findings from this laboratory are instead consistent with the idea that a dopamine agonist, such as quinpirole, acts at presynaptic heteroreceptors on glutamate terminals. The affinity of these receptors could be lower than that of dopamine autoreceptors, explaining why high doses of intra-accumbens quinpirole are necessary to decrease novelty-induced locomotion. In 10-day-old rat pups, neither novelty-dependent dopamine agonist-induced locomotor suppression nor glutamate antagonist-induced locomotor activation is exhibited. The late ontological maturation of these behaviors indicates that they require a neural mechanism that does not become functional until sometime between 10 and 20 days of age. The medial prefrontal cortex and the hippocampus mature relatively late in ontogeny, making it possible that their glutamatergic inputs to the nucleus accumbens are the late-maturing modifiers of nucleus accumbens neurotransmission. Without these glutamate inputs to the nucleus accumbens, 10-day-old rat pups would not have the hypothesized "switching" mechanism necessary to determine pre- vs. post-synaptic activity of dopamine and glutamate in the nucleus. In addition, without these glutamate inputs 10-day-old rat pups would not have the presynaptic glutamate fibers on which a dopamine agonist would act, even if the nervous system could provide another "switching" mechanism. These factors may result in the lack of adult-like responding to intra-accumbens injections of either MK801 or quinpirole.

In this manner, the neurodevelopmental approach to studying the nervous system has provided some information concerning the interaction between glutamate and dopamine in the nucleus accumbens. Nevertheless, to echo the conclusion of Pennartz in the words of Burns et al. (1994, p. 526), "simple hypotheses of glutamate-dopamine

interactions in the ventral striatum are unlikely to explain how behaviorally meaningful information is represented in the ventral striatum."

Future Experiments

As would be expected from a successful research program, the present investigations generated many more questions than answers. Thus, follow-up experiments could address several issues: What is the more precise time course of maturation for the behavioral responses to MK801 or quinpirole? Can the locomotor-potentiating interaction between MK801 and exposure to a novel environment be mediated in the nucleus accumbens? What is the course of biochemical maturation of glutamate transmission in the nucleus accumbens? Is there a relationship between ontological changes in stress hormone release and responses to novelty? What is the role of glutamate activity at other subtypes of glutamate receptor in locomotion across ontogeny?

First, whatever the mechanisms are that mediate glutamate antagonist-potentiated locomotor activation and dopamine agonist-induced locomotor suppression, they are not yet mature in the 10-day-old rat pup but do become functional by 20 days of age. Research can now focus on the identification of biochemical changes that might take place between 10 and 20 days of age to mediate the maturation of the behavior. Ten days is a relatively long period in development of the rat nervous system, however. Future experimentation might therefore define more precisely at what age the behaviors become adult-like in nature. Precise definitions of the time course of maturation might reveal whether the behaviors suddenly switch into adult-like form or whether changes in

behavior are more gradual. Correlations between brain and behavioral maturation may eventually lead to defining causal relationships between neural and behavioral events.

Second, imposing the delay between intra-accumbens MK801 injection and placement in the novel environment would show whether or not MK801 acting specifically in the nucleus accumbens could interact with exposure to a novel environment to increase the initial levels of locomotion. If it can, then further studies could focus on the nucleus accumbens with the goal of defining the synaptic mechanisms mediating the behavior. Such studies would entail studying the interaction between glutamate and dopamine by employing ligands at receptors for both neurotransmitters. Improvements on the intra-accumbens injection technique would be inherent in such a follow-up study. To minimize drug diffusion, low injection volumes would be preferable. To minimize spread into the lateral ventricles, injecting into the accumbens from an angle off the vertical would suffice.

Third, subsequent to localizing the interaction between MK801 and environmental novelty to the nucleus accumbens, ontological changes in the behaviors elicited by MK801 and environmental novelty could be correlated with changes in neural function in the nucleus accumbens. The correlation, however, requires more information on biochemical maturation in the nucleus accumbens than is presently available. Future investigations should include: retrograde labeling studies to show when the glutamate projections to the accumbens mature; microdialysis studies of glutamate release or post-mortem tissue analyses of the accumbens to explore when and in what patterns the projections release glutamate into the accumbens; receptor autoradiography to examine

changes in the density and distribution of glutamate receptors; and anterograde labeling studies to determine when the output pathways of the accumbens are in place.

Fourth, the ideas set forth in the present research concerning the role of stress hormones in responses to novelty and MK801 could be further explored. Data on stress hormone release across ontogeny do not cover a wide range of ages or behaviors, so future experiments could record changes in baseline plasma levels of stress hormones, changes in release due to exposure to novelty or other stressors, and responses of developing rats to manipulations such as adrenalectomy which block the release of stress hormones.

Finally, NMDA receptors certainly are not the only subtype of glutamate receptor to play a role in rat behavior across ontogeny. While it was necessary to limit the scope of the present research to one subtype of receptor, this procedure limited comprehension of the full potential glutamate has for modulation of behavior. Thus, parallel studies to those already conducted and those proposed above could be carried out for other subtypes of glutamate receptor.

A research program including these studies would compliment the present research in a neurodevelopmental approach to studying the role of nucleus accumbens glutamate in locomotion. Conclusions from the research would have implications for developmental psychobiology as an academic interest as well as a practical interest; nucleus accumbens glutamate has been implicated in many physiological and pathological conditions, including schizophrenia, drug abuse and Parkinson's disease.

Clinical Significance

Schizophrenia

Clinical research indicates that mesocorticolimbic feedback loops between the VTA, nucleus accumbens, cortex and hippocampus are dysfunctional in schizophrenia, a human disorder including motor and limbic abnormalities (Doran et al. 1988, Weinberger 1988, Weinberger et al. 1988). The neurotransmitters glutamate and dopamine are major determinants of activity in these pathways and, while the importance of dopaminergic dysfunction in schizophrenia has long been emphasized (Carlsson 1988), glutamate has become a new target for the etiology and treatment of schizophrenia (Halberstadt 1995, Moghaddam 1994, Olney and Farber 1995a and b, Wachtel and Turski 1990). Primary involvement specifically of the NMDA receptor has even been postulated (Farber et al. 1995, Henn 1995, Olney and Farber 1995b). Given that corticofugal glutamate release regulates subcortical neurotransmission, dysfunction in corticofugal fibers may result in excessive dopamine release to the limbic system, including the nucleus accumbens (Deutch 1992). The removal of this hypothetical filter of information feedback to the cortex may result in the classic symptomatology of limbic system damage, including inappropriate responses to stress, attentional deficits and poor performance on delayed response tasks (Carlsson 1988). Given this clinical profile, schizophrenia is most likely a developmental disease of limbic and motor dysfunction. As such, it is likely to involve a brain region known as the limbic-motor integrator, namely the nucleus accumbens.

A perplexing issue in schizophrenia is that severe symptoms of the disease do not appear until late adolescence and early adulthood. The late onset of schizophrenic symptomatology indicates that developmental abnormalities depend on maturational

changes in the nervous system to manifest the most debilitating symptoms of the disease and has led to a focus on neurodevelopmental changes associated with adolescence and early adulthood to account for the age at onset of schizophrenic symptoms (Walker 1994, for review). (Nevertheless, surprisingly little research has been carried on the periadolescent rat.) In fact, a leading theory as to the etiology of schizophrenia is that glutamate projections from the prefrontal cortex to the midbrain or from the hippocampus to the prefrontal cortex mature abnormally, but that behavioral dysfunction is not apparent until after puberty because that is when these projections are first used in the affected behaviors (Jones 1995, Keshavan 1994, Weinberger 1988). The best animal models of schizophrenic symptoms to date are those in which neonatal lesions lead to periadolescent behavioral dysfunction (Goldman-Rakic 1987, Lipska et al. 1994).

It is now apparent that schizophrenia can also entail motor dysfunction early in life (Russell 1994, Walker 1994). Thus, a broader developmental frame of reference is needed in order to incorporate the subtle signs of dysfunction apparent in preschizophrenic infants and children. For example, the disorder could be one in which cortical abnormalities present very early in ontogeny produce particular symptoms depending on which aspects of the nervous system predominate over behavior at a particular developmental stage. The sensorimotor cortex matures early and is myelinated early, so abnormalities in corticofugal projections could be expressed as motor dysfunction early in ontogeny. The limbic cortex matures later and is myelinated later, so abnormalities in those projections could be expressed as cognitive, emotional, and stress-related problems later in life. With continued aging, the predominance of limbic cortical function subsides, as do florid psychotic symptoms in elderly schizophrenics, and motor

symptoms become prevalent again. Walker speculates further that selective deficits in corticofugal glutamate transmission to the inhibitory output pathway from the striatum could result in dyskinesias of the schizophrenic type. As the dual-output pathway hypothesis was extended to the nucleus accumbens in relation to novelty-induced locomotion (Carlsson 1993, Svensson et al. 1992b, 1994), so might it be extended to the nucleus accumbens in relation to schizophrenia. Selective deficits in corticofugal glutamate transmission to the inhibitory output pathway from the nucleus accumbens could result in cognitive, emotional, and stress-related abnormalities.

It is difficult to draw parallels between developmental stages in humans and rats, but there is at least one parallel to be drawn from the present research. Walker posits that "cortical development, as well as age-related changes in neurotransmitter activity, moderates the behavioral expression of neural circuitry malfunction in schizophrenia" (Walker 1994, p. 454). Similarly, in the present studies, cortical development and age-related changes in neurotransmitter activity were hypothesized to moderate the expression of neural manipulations in the form of locomotor responses to the glutamate antagonist, MK801. The parallel lies in that the encephalization of control over subcortical function is the determining factor of ontological changes in behavior. The ontological time course for the parallel between schizophrenic symptomatology in humans and novelty-induced locomotion modulated by corticofugal glutamate questionable. Preschizophrenic motor symptoms such as abnormal hand posture and choreoathetoid movements of the upper limbs, occur most often in humans 2-3 years of age. Considering that this is often close to the time of weaning, the stage might correlate with the most robust locomotor responding to novelty in rat pups at 20 days of age. However, if the 2-3 year-old stage is

considered more importantly as the time of rapid motor development, then the stage correlates better with perhaps 10-15 days of age in the rat pup, a stage not investigated in the present study of responses to novelty and their modulation by glutamate. In schizophrenia, florid psychotic symptoms occur in late adolescence, a stage perhaps equivalent to 40-50 days of age in rats, because it is midway between puberty and adulthood. Again, characteristics of this stage in the rat are under-studied, so the effects of glutamate receptor blockade in rats of this age should be investigated to show whether or not the cortico-limbic system in the rat is especially sensitive to neural manipulations at this stage. Generally, testing animals in a wide range of ages can aid in detection of broad system changes across ontogeny and can promote analysis of neurological correlates that can best explain the entire life-course of the disease.

Finally, in terms of pharmacotherapy for schizophrenia, a glutamate agonist with selectivity for nucleus accumbens receptors on the inhibitory output pathway might be beneficial to counteract the hypothesized deficits in glutamate transmission (Carlsson and Carlsson 1990). However, given the elusive nature of the means through which glutamate's activity on the dual-outputs is regulated, a glutamate agonist that is selective enough not to cause unacceptable side-effects is a long way off.

Drug Use and Abuse

Deficient glutamate transmission due to developmental abnormalities may underlie psychotic symptoms in schizophrenics. Similarly, persistent blockade of glutamate transmission due to chronic abuse of drugs that have glutamate antagonist properties may induce schizophrenia-like psychotic symptoms in "normal" humans. Phencyclidine (known as PCP or angel dust) and ketamine (known as K) are both drugs

with potential for abuse due to their ability to produce feelings of euphoria, separation from the body, and invincibility. High doses or chronic use can result in paranoia and dysphoria, however. These drugs, like MK801, exert their glutamate antagonist effects at the PCP site inside the NMDA receptor-associated ion channel. To the extent that glutamate and dopamine systems have been shown to be interdependent for their effects on locomotion, it is additionally relevant that chronic abuse of psychomotor stimulants such as amphetamine or cocaine can also induce schizophrenic symptomatology in previously "normal" humans or can exacerbate psychosis in schizophrenics. Of developmental interest, children taking stimulants for attention deficit disorders can also exhibit dyskinesia of the type associated with preclinical schizophrenia. Whereas the motor disorders from chronic drug use are associated with striatal output systems, the reinforcing and emotional aspects of drug use are associated with the nucleus accumbens. Traditionally, the reinforcing aspects of drugs of abuse have been attributed to dopamine transmission into the nucleus accumbens, but the importance of glutamate transmission in drug abuse is increasingly being recognized (Carlezon and Wise 1996).

By defining activity at the PCP site with MK801 in the nucleus accumbens, as in the present studies, and by investigating glutamate-dopamine interactions across ontogeny, as in the proposed future experiments, a neurodevelopmental framework for drug abuse research can be generated. For example, investigations may address whether or not developmental pathologies in glutamate or dopamine transmission determine human propensity for drug abuse. Pharmacotherapy for drug abuse might then target re-establishment of a normal baseline of neural activity for an individual instead of treating the abuse of a particular drug in a palliative manner. In the course of normal maturation,

the common onset of drug use in adolescence might be correlated with changes in limbic circuitry, and might be halted by intense educational programs at the age of highest likelihood for drug use. In the emergency room, a compound that could acutely block the effects of PCP or ketamine would be beneficial in cases in which drug users might pose a danger to society with their psychotic behavior, but such acute treatments should be appropriately dosed for patients of various ages. The results of the present research show that there are ontological changes in responsivity to glutamatergic drugs. Full understanding of pharmacological activity at the PCP site across ontogeny would facilitate generation of behavioral and pharmacological therapies to meet these various needs.

Interestingly, the present results also confirm that female rats are more sensitive to blockade of the PCP site by MK801. This characteristic could explain why human females are less likely to abuse PCP or PCP-like compounds. They may be supersensitive to the drug effects and thus more susceptible to dosing beyond the euphoric effects, and into the anesthetic or dysphoric effects.

Parkinson's Disease

Parkinson's disease is generally considered to be a disease of the motor systems in the basal ganglia because the predominant symptoms are akinesia, bradykinesia, cogwheel rigidity, tremors and postural deficits. In addition to these well-described motor symptoms, the disease involves emotional and cognitive deficits (Richfield 1993). Degeneration of dopamine neurons in the substantia nigra, and perhaps the ventral tegmental area, leads to an imbalance between dopamine and glutamate transmission (Carlsson and Carlsson 1990). Pharmacological research and development related to

glutamate transmission may also prove beneficial in the treatment of other motor disorders involving the basal ganglia, such as Huntington's chorea. Given these clinical and neurological characteristics, disruption of the interface between motor and limbic function is indicated and successful treatment requires knowledge about the normal function of limbic-motor circuitry.

Potentiation of dopamine transmission has long been the target of pharmacological therapy for Parkinson's disease, but experiments such as the present studies point out the ability of glutamate transmission to modulate motor activity. Therefore, a new focus for pharmacological treatment could be glutamate transmission. Carlsson and Svensson (1990) suggest that MK801 might even be combined with a catecholamine agonist for maximal therapeutic effectiveness in stimulating motor activity. Yet, as mentioned above, the present studies also point out the complexities of glutamate's role in locomotion, as in the dual function of glutamate at the excitatory and inhibitory output pathways from the striatum. Therefore, extensive research is necessary for development of the optimal pharmacotherapy. Eliminating the potential side effects of NMDA antagonists, i.e. increasing locomotion without inducing stereotypies or ataxia, would need to be a focus of such research and could be addressed by closely attending to the behavioral syndrome elicited by new compounds in experimental subjects.

Parkinson's disease affects men and women with approximately equal frequency. Therefore, any pharmacological treatment must be effective for both genders. The gender-dependent effects of MK801 caution against the assumption that glutamatergic drugs will be equally effective in male and female patients, especially if females are in simultaneous estrogen replacement therapy.

Conclusion

The present research has shown that endogenous glutamate plays an integral role in translating the stimulus of a novel environment into locomotor behavior. The translation is hypothesized to involve interactions between dopamine and glutamate which modify locomotor output from the nucleus accumbens in an age- and gender-dependent manner. Understanding nucleus accumbens circuitry may lead to better diagnosis and treatment of schizophrenia, drug abuse and Parkinson's disease. Investigating the role played by glutamate in the locomotor behavior of developing and adult rats will help to provide a neurodevelopmental foundation for this understanding.

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BIOGRAPHICAL SKETCH

The author was born on June 11, 1970, in Bellefonte, Pennsylvania. She attended the University of Pennsylvania, where she began work in psychology as an assistant in the laboratory of Dr. Harvey J. Grill. In 1990, she participated in the "Research Experience in Psychology" program at the Pennsylvania State University, a National Science Foundation program that enabled her to design and implement experiments, under the mentorship of Dr. Paul Cornwell. Later that year, she travelled on the University of Pittsburgh's Semester at Sea, a one-hundred-day voyage around the world with stops at eleven foreign ports and classes conducted on-board a ship. After she was graduated from the University of Pennsylvania in 1991 with a major in psychology, she served briefly as a laboratory technician for Dr. Alan C. Spector. Her graduate training took place at the University of Florida in the Department of Psychology. Her advisor was Dr. Carol Van Hartesveldt and other mentors included Dr. Mark Lewis in Psychiatry, Dr. Joanna Peris in Pharmacodynamics, Dr. Neil Rowland in Psychology, and Dr. Alan Spector in Psychology. Much of her research at the University of Florida was supported by funding from the National Institutes of Health through a Center for Neurobiological Sciences Traineeship. As a Fulbright Scholar in 1995 and 1996, the author conducted research in the laboratory of Dr. Urban Ungerstedt at the Karolinska Institute in Stockholm, Sweden.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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